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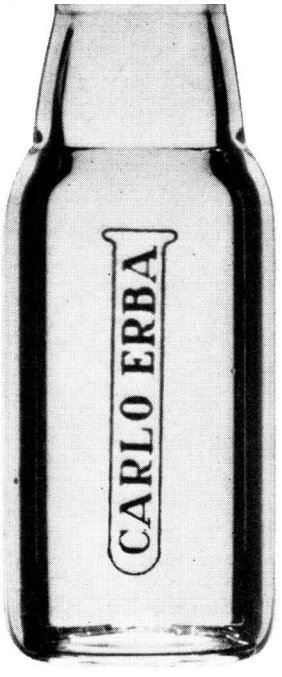

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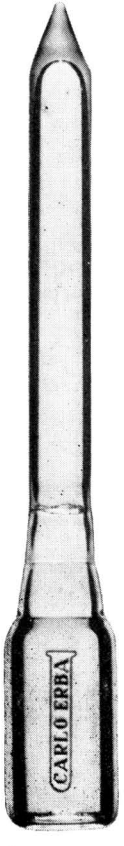

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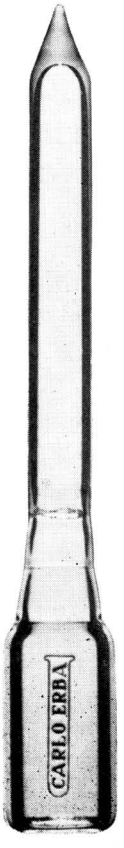

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THE ANALYST

Summaries of Papers in this Issue

Controlled-potential Coulometers Based upon Modular Electronic Units

Part I. Development of Equipment

The construction of controlled-potential coulometric equipment is described in which modular electronic units are used. A digital voltmeter is used with the equipment for measuring and integrating the electrolysis current. It is readily adaptable and can be used manually or automatically, with a sample changer and print-out unit.

G. PHILLIPS and G. W. C. MILNER

Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Near Didcot, Berkshire.

Analyst, 1969, **94**, 833–839.

Controlled-potential Coulometers Based upon Modular Electronic Units

Part II. The Determination of Ruthenium by Controlled-potential Coulometry

A method is described for the determination of ruthenium by controlled-potential coulometry. Quadrivalent ruthenium as the binuclear $[Ru_2O]^{6+}$ chlorocomplex in 5 M hydrochloric acid is reduced to tervalent ruthenium at the platinum electrode at a potential of +0·05 V *versus* the S.C.E. Milligram amounts of ruthenium can be determined with a coefficient of variation of 1·0 per cent. The method has been applied to uranium - ruthenium alloys and uranium carbide - ruthenium cermet materials.

G. WELDRICK, G. PHILLIPS and G. W. C. MILNER

Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Near Didcot, Berkshire.

Analyst, 1969, **94**, 840–843.

Pre-concentration of Carbonyl Compounds from their Medium Followed by Polarographic Determination of their Azomethine Derivatives

A method for the pre-concentration of carbonyl compounds on a chromatographic column via the formation of an azomethine derivative is described. The subsequent elution of the derivative is monitored polarographically.

M. D. BOOTH and B. FLEET

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1969, **94**, 844–846.

Some Observations on the Analytical Usefulness of Electrochemiluminescence for the Determination of Microgram Amounts of Aromatic Hydrocarbons

Measurement of the electrogenerated chemiluminescence of twenty-four aromatic compounds in dimethylformamide solution has been investigated as a potential analytical technique for their determination. The emission spectra, calibration results and limits of determination are presented. Experimental requirements and optimum conditions for the electrogeneration of the light-emitting species are described.

B. FLEET, P. N. KELIHER, G. F. KIRKBRIGHT and C. J. PICKFORD

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1969, **94**, 847–854.

Voltammetric Studies with Different Electrode Systems
Part IV. Determination of Silver by Using a Silver - Molybdenum System

Silver is determined at trace level (1 to 100 p.p.m.) in organic solvents and alkalis by using a semi-micro cell incorporating a silver - molybdenum system. The determination is carried out on a differential cathode-ray polarograph. With this electrode system only silver and mercury cations were reduced and the peak potentials were well separated. No chemical separations are involved. Factors affecting the silver reduction in organic solvents are discussed.

V. T. ATHAVALE, M. R. DHANESHWAR and R. G. DHANESHWAR
Analytical Division, Bhabha Atomic Research Centre Modular Laboratories, Trombay, Bombay-74, India.

Analyst, **1969**, **94**, 855–859.

Limit of Determination in Photometric Titrations with Self-indicating Systems

An attempt is made to predict the lower limit of concentration (the limit of determination) of a photometric titration based on a self-indicating reaction. When only instrumental factors influence the precision of the result of such a titration it appears to be possible to express the limit of determination in terms of the precision of the absorbance reading and other characteristics of the apparatus (path length and cell volume) and of the reaction ($\Sigma\epsilon$).

The theoretical prediction has been checked by using two commercially available photometers and two reactions, *viz.*, the titration of vanadium(IV) with cerium(IV), and the titration of cerium(IV) with iron(II). The agreement between the theoretically predicted value of the limit of determination and the experimental value appeared to be satisfactory.

E. R. GROENEVELD and G. den BOEF
Laboratory for Analytical Chemistry, University of Amsterdam, The Netherlands.

Analyst, **1969**, **94**, 860–863.

The Atomic-emission Spectroscopy of the Rare Earth Elements in a Separated Nitrous Oxide - Acetylene Flame

Flame emission in the interconal zone of a separated nitrous oxide - acetylene flame is shown to provide a sensitive technique for the detection of the rare earth elements, scandium and yttrium. The relative intensities of the principal atomic lines of these elements emitted in this flame have been measured, and the detection limits at the lines most suitable for flame-emission spectroscopy are reported for aqueous and ethanolic sample solutions. The effect on the atomic-emission intensities obtained for each of the sixteen elements investigated in the presence of the other fifteen elements has been investigated. At the concentrations used in this general survey no serious spectral line interferences between the rare earths were observed because of the simple spectra excited by the hot, reducing fuel-rich flame.

D. N. HINGLE, G. F. KIRKBRIGHT and T. S. WEST
Chemistry Department, Imperial College, London, S.W.7.

Analyst, **1969**, **94**, 864–870.

Emission Spectra Obtained from the Combustion of Organic Compounds in Hydrogen Flames

A study is presented of the emission spectra produced by nebulisation of organic liquids into a nitrogen - hydrogen diffusion flame burning in air, and into a laminar-flow pre-mixed air - hydrogen flame. Both flames exhibit low background emissions and that of the diffusion flame is particularly low even over the OH-band region. Carbon, hydrogen and oxygen-containing compounds exhibit intense bands for CH, CHO, C_2 and OH species. Nitrogen-containing organic compounds additionally display NH, NO and CN bands; chlorine-containing compounds give CCl bands in the diffusion flame; sulphur compounds give CS and S_2 bands; and phosphorus compounds give HPO and PO bands.

The spectral distribution of these bands and their intensities indicate that many types of organic compound can be characterised and identified by direct observation of their emission spectra during combustion in these cool flames.

R. M. DAGNALL, D. J. SMITH, K. C. THOMPSON and T. S. WEST
Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1969, **94**, 871–878.

A Method for the Determination of Lead in Blood by Atomic-absorption Spectrophotometry

A method for the determination of trace amounts of lead in blood is described. The organic material in the blood is oxidised by dry ashing at 500° C and a solution of the ash in dilute hydrochloric acid prepared. The lead in the ash solution is determined by atomic absorption at 217 nm in an air - propane flame after isolation by a double-extraction procedure with dithizone and ammonium tetramethylenedithiocarbamate as complexing agents. Recovery tests are carried out and the lead content of the blood of workers from a lead mine, determined by this method, is compared with results obtained by using the mixed colour method of the Analytical Methods Committee. Comparative tests with wet-oxidation and dry-ashing techniques are made on samples of blood to which known amounts of lead have been added. The interference caused by bismuth is also investigated.

P. P. DONOVAN and D. T. FEELEY
Public Analyst's Laboratory, Galway Regional Hospital, Galway, Ireland.

Analyst, 1969, **94**, 879–883.

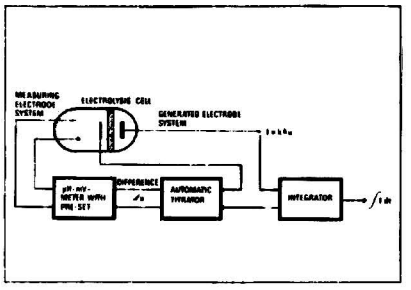
Atomic-absorption Determination of Strontium in a Standard Plant Material: Comment on Results of Inter-laboratory Comparison

It is claimed that the concentration of strontium in Bowen's standard kale (*Analyst*, 1967, **92**, 124) is 101·13 ± 2·38 p.p.m., on evidence that X-ray fluorescence values obtained subsequently to Bowen's inter-laboratory comparison were in good agreement at 101 p.p.m. with atomic-absorption values obtained in this laboratory by using anion exchange and the addition method, but not reported separately from other atomic-absorption results by Bowen. Methods used in obtaining the neutron-activation results and these other atomic-absorption results implied in the inter-laboratory comparison should be examined for serious interferences.

D. J. DAVID
Division of Plant Industry, CSIRO, Canberra, A.C.T., Australia.

Analyst, 1969, **94**, 884–885.

Block diagram of Coulometric Analyzer



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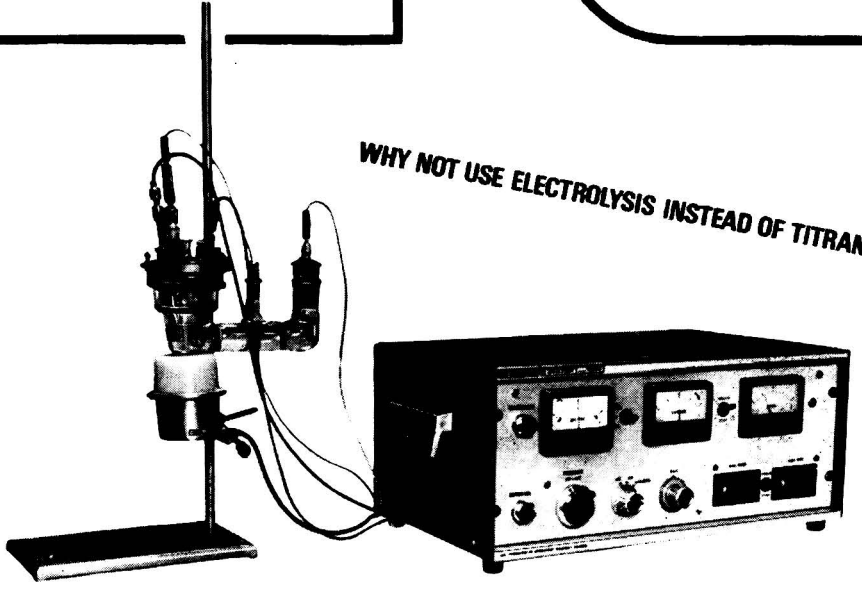


WHY NOT USE ELECTROLYSIS INSTEAD OF TITRANT

OCTOBER, 1969 Vol. 94, No. 1123

THE ANALYST

Controlled-potential Coulometers Based upon Modular Electronic Units

Part I. Development of Equipment*

BY G. PHILLIPS AND G. W. C. MILNER

(Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Near Didcot, Berkshire)

The construction of controlled-potential coulometric equipment is described in which modular electronic units are used. A digital voltmeter is used with the equipment for measuring and integrating the electrolysis current. It is readily adaptable and can be used manually or automatically, with a sample changer and print-out unit.

THE construction of new electronic controlled-potential coulometers has been reported by several authors,[1,2,3,4] following the original paper by Booman[5] in 1957. In each case the instrument is designed to perform two basic functions during the electrolysis. Firstly, it maintains the potential of the working electrode at a selected value with reference to a standard electrode in contact with the solution, and, secondly, it integrates the electrolysis current passing during each determination. The most commonly used integrator takes an analogue proportion of the electrolysis current and integrates this in a resistance - capacity circuit in conjunction with an operational amplifier. This amplifier is a critical component of the coulometer, and needs careful maintenance to ensure low drift. It has the disadvantage of going out of balance when the coulometer is overloaded. In a more recent alternative integrator an a.c. frequency converter is used to convert a d.c. voltage into an a.c. signal, which is measured with an electronic counter.[6] An instrument based on this principle has been built and tested at A.E.R.E., Harwell, and details are given in this paper. Successful applications of this instrument in analysis are reported in Part II (page 840).

DESCRIPTION OF THE COULOMETER—

The development of digital methods of data handling and the need for flexibility in the construction of complex experimental equipment has led to the availability of comprehensive unitised systems of electronic equipment. One such system is the Harwell-designed "2000 series" of electronic units.[7] A selection of these units, together with a coulometer unit built to the same design principles and a Solartron digital voltmeter, have been used to construct a controlled-potential coulometer.

A circuit diagram of the coulometer unit (2220) is shown in Fig. 1. The three-electrode electrolysis cell is shown schematically on the extreme left of the diagram. The potentiostat compares the potential difference between the working electrode and the reference electrode with that of an adjustable reference potential derived from a zener diode circuit (designated "set volts" in Fig. 1). In operation any difference between these two potentials is adjusted to zero by a current passing through the electrolysis cell via the working electrode and counter electrode. This current passes through a 100-Ω precision resistor, which is connected in the input circuit of the digital voltmeter. The other circuits in the coulometer unit comprise a timing circuit to control the sampling rate of the digital voltmeter and an on - off system operated by a manual remote relay.

A unit block diagram of the controlled-potential coulometer in the manually operated form, showing signal connections, is given in Fig. 2. The five "2000 series" units used fit on a shelf, which can be accommodated within a standard 19-inch rack. The coulometer unit 2220 has three operational modes, *viz.*, "set volts," "stand by" or "remote," and "operate."

* Paper presented at the Second SAC Conference 1968, Nottingham.

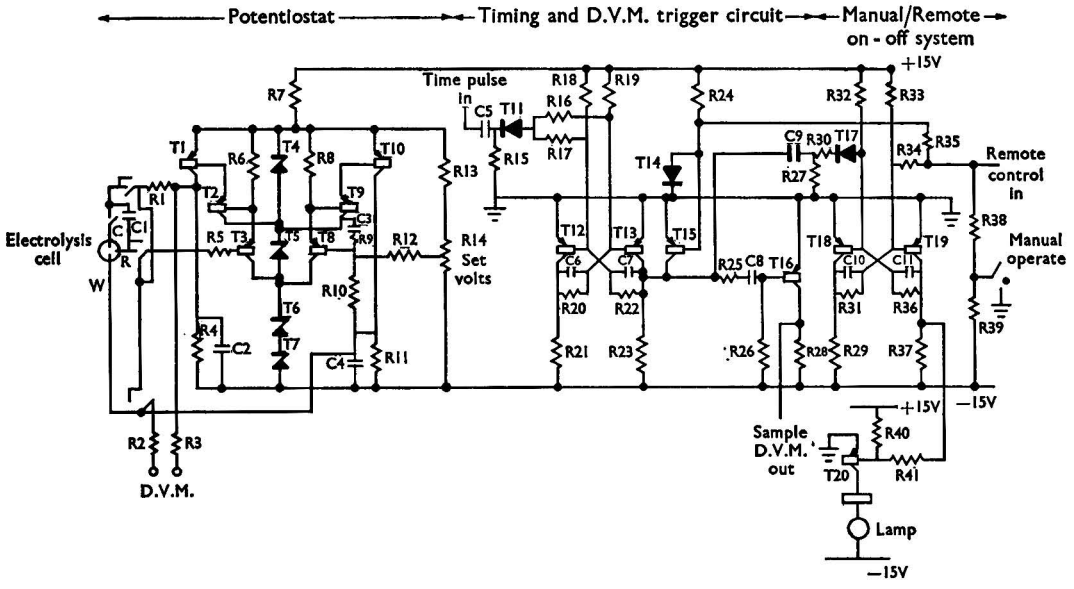


Fig. 1. Circuit diagram of the coulometer unit 2220: W, working electrode (platinum, mercury or silver); C, counter electrode (usually platinum); R, reference electrode; and D.V.M., digital voltmeter (for values of components see Appendix I)

In the "set volts" position the required working potential is set by adjustment of a 10-kΩ helipot on the front of the unit. The value of this potential is displayed on the digital voltmeter by using the "auto" operational mode. In the "operate" position the coulometer

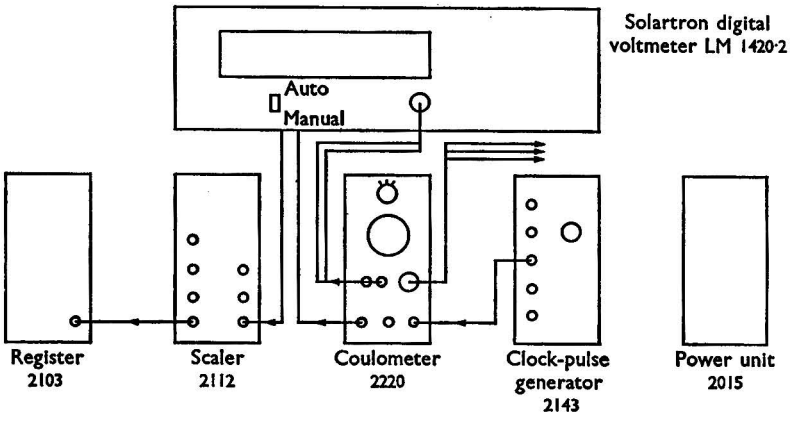


Fig. 2. Block diagram of the controlled-potential coulometer

unit 2220 receives a train of pulses from the clock-pulse generator 2143 at a rate of 5 s^{-1}. The pulses are inverted and used to operate the "remote sample" of the digital voltmeter at a rate of 2·5 s^{-1}. The electrolysis is initiated with the first pulse received after switching on and is continuous until the coulometer unit is switched to the "stand by" position. The electrolysis current passes through a 100·0-Ω precision resistor in the coulometer unit, the potential drop across which is sampled by the digital voltmeter. Sampling of the electrolysis current commences with the second pulse received after switching on and subsequently with every other pulse until the coulometer unit is returned to the "stand by" position. A count

corresponding to the integral of the area under the electrolysis current - time graph is accumulated serially in the scaler 2112 via a control and gate circuit connected to the rear of the digital voltmeter (Fig. 3). The scaler records four times the number of digits indicated on the digital voltmeter display. In operation, therefore, the digital voltmeter displays current readings, which can be used to follow the course of the electrolysis visually, and the scaler displays the current integral. With the digital voltmeter on the 2-V range, a reading of 1 V corresponds to a current of 10 mA, and a reading of 0·001 V to a background current of 10 μA. The digital voltmeter can be calibrated internally against a standard cell and the coulometer is, therefore, inherently accurate and, with the setting described above, gives 10^6 counts C^{-1}. Any change in the range setting of the digital voltmeter, or of the value of the precision resistor, or of the sampling rate set by the clock-pulse generator, produces a *pro rata* change in the calibration value. With the above settings the scaler can accumulate 2×10^6 counts or about 20 micro-equivalents; the overflow register shown is necessary only above this level. It is essential for accurate results and optimum precision to match the electrolysis current to the dynamic range of the digital voltmeter. On the 2-V range this is from 22·99 mA to 10 μA. The range can be extended in the upward direction by using a 50·0-Ω precision resistor in the electrolysis circuit, and in the downward direction by the range settings on the digital voltmeter.

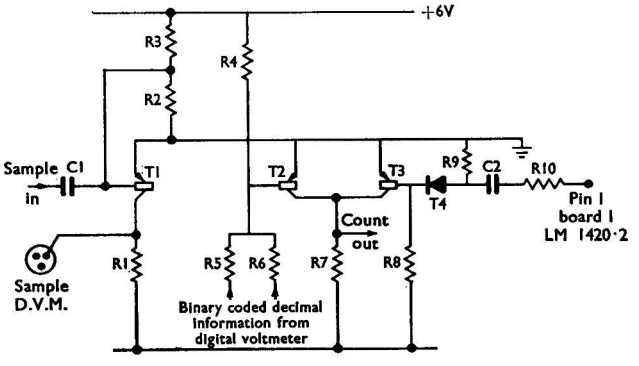


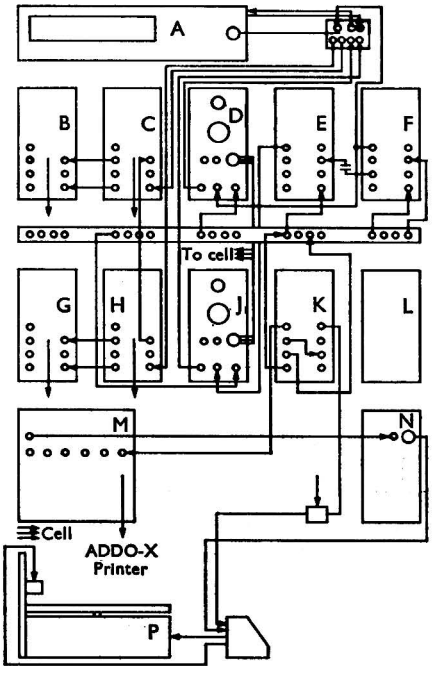
Fig. 3. Control and gate circuit LM 1420.2 (for values of components see Appendix II)

AUTOMATIC OPERATION—

Controlled-potential coulometric titrations are normally terminated when the current has fallen to a stable background level of at least three orders of magnitude lower than the initial current. For a given reaction, the time required to reach this level is dependent mainly upon the surface area of the working electrode, the volume of electrolyte and the rate of stirring and, to a much lesser extent, upon the temperature and the concentration of the material being electrolysed. If the parameters of the electrolysis cell are fixed, it is feasible to terminate a controlled-potential coulometric titration after a fixed time. The coulometer unit has been designed to operate either manually or by means of suitable binary levels when set in the "stand by" position. These levels can be obtained from a "2000 series" pre-set scaler 2166 connected to receive pulses from the clock-pulse generator and to give a 5-V negative-going level at the end of the set time. The working potential is set manually as described above and the coulometer unit switched to the "stand by" position. The electrolysis time required to reach the background current level is then set on the pre-set scaler 2166. Electrolysis is initiated by means of the re-set button on the scaler 2166, which continues to operate until the pre-set time has elapsed. The equipment then switches off leaving the current integral displayed on the scaler 2112.

A controlled-potential coulometric determination is usually a two-step process, consisting of a pre-electrolysis at one potential, to condition the electrolyte, to remove an interfering reaction or to adjust the valency of the species of interest, followed by a change of potential to enable the electrolysis of the required constituent to be carried out. A fully automatic coulometer must, therefore, be able to apply two different pre-set potentials to the working

electrode in a timed sequence. This can be achieved by arranging two single-stage automatic units to operate in sequence, as shown in Fig. 4. The power unit and clock-pulse generator are common to both stages, and a small switching unit is required to switch the operation of the digital voltmeter from the first coulometer unit to the second. The sequence of operations is initiated by using the re-set button of register No. 1, which re-sets all integrating scalers and the pre-set timing scaler No. 1. Coulometer No. 1 commences to operate, and electrolysis at the first selected potential continues for N units of time set on pre-set scaler No. 1. After that time coulometer No. 1 is switched off, and pre-set scaler No. 2 is re-set automatically by the $(N + 1)$th pulse. Coulometer No. 2 then commences to operate at the second selected potential for N' units of time set on pre-set scaler No. 2. Coulometer No. 2 is switched off at N' and electrolysis ceases, leaving the integral of each electrolysis step displayed on the corresponding scalers.



A = Digital voltmeter LM 1420.2
B = Integrating scaler 2117 (1a)
C = Integrating scaler 2117 (1)
D = Coulometer (1)
E = Pre-set scaler 2166 (2)
F = Pre-set scaler 2166 (1)
G = Integrating scaler 2117 (2a)
H = Integrating scaler 2117 (2)
J = Coulometer (2)
K = Gated clock-pulse generator 2166
L = Power unit 2015
M = Print-out control unit 2140
N = Stirrer control
P = Sample changer

Fig. 4. Automatic controlled-potential coulometer with sample changer

The equipment can also be arranged to work in conjunction with a printer and sample changer (Fig. 5) with the additional units shown in Fig. 4. The cycle of events is initiated manually by means of the start button on the print-out control unit 2140. This prints and re-sets the integrating scalers and triggers and sample-changing mechanism. The sample-changing mechanism raises the stirrer, gas line and electrode assembly clear of the electrolysis cell and rotates the next cell into position. The electrode assembly and attachments are then lowered into the new solution. The mechanism can be set to perform this operation once per cycle, or twice to permit rinsing of the electrode assembly. The time between the completion of one electrolysis and the re-setting of pre-set scaler No. 1, which initiates the next

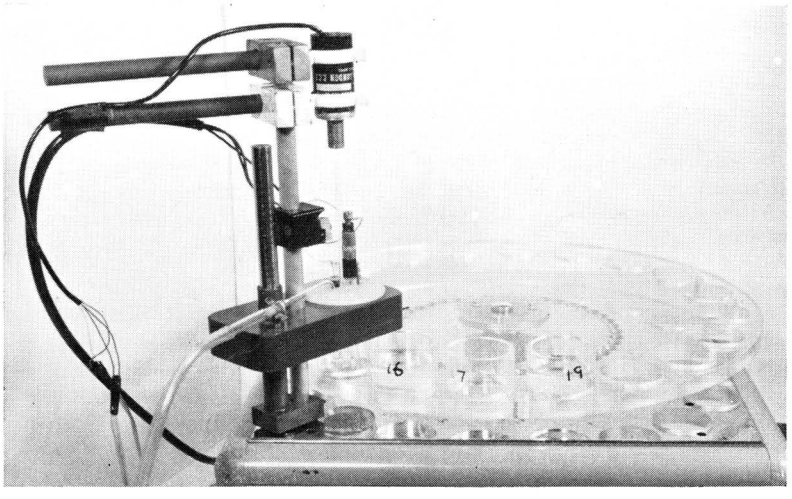


Fig. 5. Sample changer

[To face page 836

electrolysis, can be varied up to a maximum of $6\frac{2}{3}$ minutes. The time required for the mechanical movements is about 1 minute or less; the additional delay allows time for the removal of oxygen from the solution. The turn-table holds twenty electrolysis cells, and hence twenty automatic coulometric titrations can be carried out on irreversible systems not requiring a rinsing step, or ten on reversible systems needing rinsing of the electrodes. In this application the scaler units are changed to type 2117 to allow print-out access, and the gated clock-pulse generator 2116 is substituted for the 2143 to permit a delay to be introduced between determinations.

ELECTROLYSIS CELLS—

(*a*) *Manual sample changing*—Two types of electrolysis cells have been used with the above coulometer, one with a platinum-gauze working electrode, the other with a mercury-pool electrode. The cell for use with a platinum-gauze working electrode was of the type previously described,[2] in which cation or anion-exchange membranes (Permutit C20 or A20) were used to separate the three compartments. In practice it was found that leaks rapidly developed from the edges of the membranes because of attack by hydrochloric acid on the polystyrene cement used to seal the edges. This was replaced with a small circular gasket cut from $\frac{1}{16}$-inch thick neoprene sheet with cork borers. The cell for use with a mercury pool was of the type described by Jones, Schults and Dale.[8]

(*b*) *Automatic sample changing*—The cell used in the automatic sample changer for controlled-potential electrolysis with a platinum electrode or a mercury pool consists of a Perspex open-topped cylinder, $1\frac{1}{2}$ inches i.d. and $1\frac{1}{2}$ inches high. The outside of the cylinder is ledged to locate the cell in holes around the perimeter of the turn-table. The stirrer, gas inlet and electrode assembly are accommodated in a polythene cover which, in the lowered position, rests on top of the electrolysis cell. Possible damage to the electrolysis cells or electrode assembly by overdriving is avoided by means of a slipping clutch in the drive mechanism. When used with a mercury-pool electrode the cell requires 8 ml of mercury and the same volume of solution. For use with a platinum-gauze electrode a solution volume of 14 ml is required.

INSTRUMENTAL OPERATIONAL PROCEDURES—

(*a*) *Electrolysis at a controlled potential*—Switch the digital voltmeter to "auto" operation on the 2-V range, and the coulometer unit to "set volts." Adjust the potentiometer control on the coulometer unit until the required working potential is displayed on the digital voltmeter. During this procedure random counts are recorded by the scaler and can be ignored. Switch the coulometer unit to "stand by" and the digital voltmeter to "manual" operation. Clear the scaler and register, and switch the coulometer to "operate." The initial voltage readings are within the range of the digital voltmeter, *i.e.*, less than 2·300 on the 2-V range. Allow the electrolysis to proceed until a stable background current is achieved, then switch the coulometer unit back to "stand by." Note readings on the scaler and register, and calculate the weight of material electrolysed as follows—

$$W = \frac{N \times A \times F}{n \times 96{,}487},$$

where W is the weight of material electrolysed, g; N, the scaler and register readings; A, the atomic weight; F, the coulometer factor, C counts^{-1}; and n is the number of electrons involved in the reaction.

(*b*) *Determination of the E_o' of a reversible redox system*—The necessary results for the construction of a coulogram and determination of the E_o' of a reversible redox system can be obtained by using the procedure described in (*a*) repetitively at intervals of working potential over the critical range. This is time consuming, as it is necessary to wait for equilibrium to be achieved at each applied potential value. The process can be speeded up by using the following procedure.

Switch the digital voltmeter to manual operation, clear the scaler and register and switch the coulometer to operate. Adjust the potentiometer control on the coulometer unit until electrolysis commences at a slow rate, *e.g.*, about 10 per cent. of the maximum rate. Allow electrolysis to proceed for about 1 minute, then carefully reverse the potential applied until the electrolysis rate is reduced to an insignificant level, or stops completely. Switch

the scaler off and note the reading. Switch the coulometer unit to "set volts" and the digital voltmeter to "auto," and note the applied potential. Return the coulometer unit to "stand by" and the digital voltmeter to "manual," and switch on the scaler. Switch the coulometer to "operate," and repeat the above process until no further electrolysis is obtained on increasing the applied potential.

Plot the counts recorded against the applied potential or plot

$$\log \text{Count} \equiv [\text{oxidised}] \div \text{Count} \equiv [\text{reduced}]$$ against the applied potential

and read off the E_0' value.

(*c*) *Calibration check*—With the digital voltmeter accurately calibrated on the 2-V range and the clock-pulse generator set to deliver pulses at 200-millisecond intervals the coulometer should record 1 count per 10^{-6} C, or at 100-millisecond intervals 1 count per 5×10^{-7} C. This condition can be readily confirmed by substituting a 1·35-V mercury cell and 100·0-Ω precision resistor for the electrolysis cell, as shown in Fig. 6. On switching the coulometer unit to "operate" and allowing a few minutes for stabilisation, a constant current is obtained. The count accumulated in a fixed interval of time can then be noted and related to the number of coulombs passed. This procedure can be repeated at various current levels by adjustment of the potentiometer on the coulometer unit. This calibration check can be carried out manually or automatically. In the latter case, however, it is better to transfer time control to the scaler 2112, leaving the coulometer in continuous operation in order to avoid transient effects on switching on or off.

This calibration procedure has been carried out repeatedly with a precision of $\leqslant 0{\cdot}03$ per cent. (coefficient of variation) and an accuracy within the same limits.

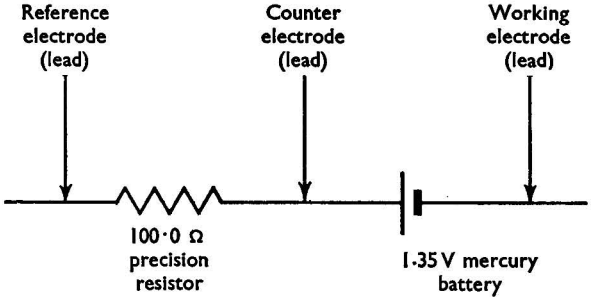


Fig. 6. Circuit for calibration check

(*d*) *Test of equipment with standard solutions*—The equipment has been tested with standard solutions of iron, uranium, plutonium and copper. The results obtained are shown in Table I.

TABLE I

RECOVERIES OBTAINED WITH STANDARD SOLUTIONS

Weight taken, mg	Weight found, mg	Precision (coefficient of variation), per cent.	Degrees of freedom
Iron (Specpure), 1·592 ..	1·589	0·15	6
Uranium, 9·580 ..	9·585	0·18	8
Plutonium, 2·058 ..	2·059	0·10	5
Uranium, 1·302 ..	1·303	0·44	9
Copper, 1·009 ..	1·009	0·20	8
*Uranium, 4·864 ..	4·859	0·34	11
*Iron, 1·446 ..	1·442	0·18	11

* With the automatic sample changer.

DISCUSSION

The use of a modular system of construction for controlled-potential coulometric equipment has a number of advantages. The system can be designed by the user to be operated manually, semi-automatically or completely automatically to suit the nature of the work to be carried

out. The method of integration used is free from the overloading difficulties associated with operational amplifier integrators used in most coulometric equipment. Skilled electronic knowledge is not required by the user, and faults can be rapidly identified and overcome by unit replacement. The equipment is more suitable for routine operation but can be used manually for the development of analytical controlled-potential coulometric procedures. The current limit of the equipment is 46 mA and it is most suitable for determinations in the region of 20 micro-equivalents. There is no instrumental lower limit other than that set by background current effects.

Appendix I

List of components for Fig. 1

R_1	= 100-Ω ± 0·1 per cent. precision resistor
R_2, R_3	= 1-MΩ resistors
R_4, R_{11}, R_{25}	= 470-Ω resistors
R_5	= 8·2-kΩ resistor
R_6, R_8, R_{18}, R_{19}, R_{24}, R_{32}, R_{33}, R_{40}	= 100-kΩ resistors
R_7	= 100-Ω, 1-W wire-wound resistor
R_9, R_{10}, R_{26}, R_{38}	= 10-kΩ resistors
R_{12}	= 39-kΩ resistor
R_{13}, R_{21}, R_{23}, R_{29}, R_{37}	= 2·7-kΩ resistors
R_{14}	= 10-kΩ helipot resistor
R_{15}, R_{27}, R_{30}, R_{39}	= 4·7-kΩ resistors
R_{16}, R_{17}	= 2·2-kΩ resistors
R_{20}, R_{22}, R_{31}, R_{36}	= 18-kΩ resistors
R_{28}	= 1·5-kΩ resistor
R_{34}, R_{35}, R_{41}	= 22-kΩ resistors
C_1	= 0·1-μF capacitor
C_2, C_4	= 1-μF capacitors
C_3	= 0·01-μF capacitor
C_5, C_8	= 150-pF capacitors
C_6, C_7, C_9, C_{10}, C_{11}	= 56-pF capacitors
T_1, T_{10}	= BCY31 transistors
T_2, T_3, T_8, T_9	= C111 transistors
T_4, T_5, T_6, T_7	= OAZ240 transistors
T_{11}, T_{14}	= ASZ13 transistors
T_{12}, T_{13}, T_{15}, T_{18}, T_{19}	= ASZ21 transistors
T_{16}	= 2N1309 transistor
T_{17}	= AAY11 transistor
T_{20}	= ACY19 transistor

Appendix II

List of Components for Fig. 3

R_1	= 4·7-kΩ resistor
R_2	= 12-kΩ resistor
R_3	= 27-kΩ resistor
R_4	= 8·2-kΩ resistor
R_5, R_6	= 18-kΩ resistors
R_7	= 3·3-kΩ resistor
R_8	= 47-kΩ resistor
R_9	= 10-kΩ resistor
R_{10}	= 470-Ω resistor
C_1	= 390-pF capacitor
C_2	= 47-pF capacitor
T_1, T_2, T_3	= 2N1309 transistors
T_4	= AAY11 transistor

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Controlled-potential Coulometers Based upon Modular Electronic Units

Part II. The Determination of Ruthenium by Controlled-potential Coulometry*

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A method is described for the determination of ruthenium by controlled-potential coulometry. Quadrivalent ruthenium as the binuclear $[Ru_2O]^{6+}$ chlorocomplex in 5 M hydrochloric acid is reduced to tervalent ruthenium at the platinum electrode at a potential of +0·05 V *versus* the S.C.E. Milligram amounts of ruthenium can be determined with a coefficient of variation of 1·0 per cent. The method has been applied to uranium - ruthenium alloys and uranium carbide - ruthenium cermet materials.

The possibility of determining ruthenium by controlled-potential coulometry resulted from an observation in this laboratory that ruthenium interfered in the coulometric determination of uranium. Hydrochloric acid solutions appeared to be the most suitable for further investigation because the chlorocomplexes of ruthenium have been characterised and information is available on the absorption spectra of these complexes.[1] At the commencement of this study no references were available in the literature on the determination of ruthenium by coulometry, but during the course of this work Stenina, Krylov and Agasyan published controlled-potential[2] and constant-current coulometric[3] methods for ruthenium. The work of these authors is based, however, upon a mononuclear chlorocomplex of ruthenium, different from that used in our work.

Experimental

Coulometric investigation of ruthenium in chloride media—

Preparation of solutions—Ruthenium solutions, suitable for coulometry, can be prepared by alkaline fusion of ruthenium metal followed by extraction of the cooled melt with hydrochloric acid. For this purpose 100-mg amounts of Specpure ruthenium sponge were fused at 600° C with a mixture of sodium peroxide and sodium hydroxide in an alumina crucible. The cooled melt was extracted into 5 M hydrochloric acid. This solution, which was brown, gave the absorption spectrum shown in curve D of Fig. 1, with peaks at 385 and 470 nm. This spectrum is typical of the quadrivalent ruthenium polynuclear $[Ru_2O]^{6+}$chlorocomplex, but the presence of minor amounts of other ruthenium chlorocomplexes could not be ruled out solely on spectrophotometric evidence.

Coulometry at a mercury electrode—Aliquots of the above solution containing about 1 mg of ruthenium were transferred to a coulometric cell containing a stirred mercury electrode. It was found that the solution could be readily reduced and the brown colour discharged at potentials of about 0 V *versus* the S.C.E. This reduction corresponded to a one-electron change, but was not reversible within the range of application of the mercury-pool electrode. The reduced solution, which was colourless, had the absorption form shown in curve E of Fig. 1, and was typical of ruthenium(III). Further reduction of the colourless solution was possible at potentials more negative than 0 V *versus* the S.C.E., producing an intense blue colour, but the reaction did not proceed at 100 per cent. current efficiency, presumably because of the discharge of hydrogen ions. The reduction was reversible at the mercury pool, but it was not suitable for quantitative work. This preliminary work with the mercury-pool electrode indicated that further studies on the coulometry of ruthenium in chloride solutions would be more conveniently carried out with a platinum electrode.

Coulometry at a platinum electrode—Aliquots of the above solution containing about 1 mg of ruthenium were transferred to a coulometer cell containing a stationary platinum-gauze working electrode. Coulograms for ruthenium were plotted over the acidity range from 1 to 7 M hydrochloric acid. It was found that the reactions were more rapid in the more

* Paper presented at the Second SAC Conference 1968, Nottingham.

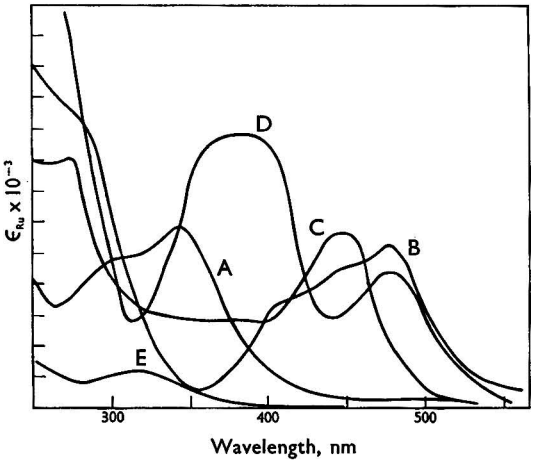

Fig. 1. The absorption spectra for chlorocomplexes of ruthenium(III), (IV) and (VI): A, ruthenium(VI), RuO_2^{2+}; B, ruthenium(IV), Ru^{4+}; C, ruthenium(IV), $Ru_2O_2^{4+}$; D, ruthenium(IV), Ru_2O^{6+}; and E, ruthenium(III), Ru^{3+}

concentrated acid solution, but with 7 M hydrochloric acid the cation-exchange membrane separators between the electrolysis cell compartments were attacked. With 5 M hydrochloric acid, however, less difficulty was experienced and the reactions were sufficiently rapid to be useful for the analysis. In 5 M hydrochloric acid the ruthenium(IV) $[Ru_2O]^{6+}$ chlorocomplex

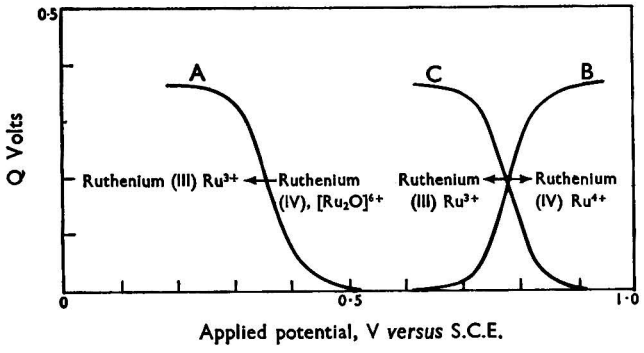

Fig. 2. Coulograms of ruthenium in 5 M hydrochloric acid

was readily reduced, with an E_0' of 0·365 V *versus* the S.C.E. (curve A, Fig. 2), giving a colourless solution. This reaction was not reversible, but re-oxidation to a brown coloured solution could be achieved at a much higher potential with an E_0' of 0·805 V *versus* the S.C.E. (curve B, Fig. 2). This second reaction was reversible (curve C, Fig. 2). It was apparent from these reactions that the ruthenium species in the second brown solution, obtained by electrochemical oxidation of Ru^{3+}, was not the same as the species in the original solution. An absorption spectrum on the second brown solution confirmed the presence of Ru^{4+} (curve B, Fig. 1). The coulometric measurements indicated that all of these reactions involved one-electron changes, but the reversible Ru^{3+} - Ru^{4+} change was not faster than the irreversible reduction of the ruthenium(IV) $[Ru_2O]^{6+}$ chlorocomplex, which could be achieved in 45 minutes in a 10-ml cell, or 30 minutes in a 5-ml cell.

From these investigations a method for determining ruthenium, based upon fusion of the sample in alkaline peroxide, extraction into 5 M hydrochloric acid and a controlled-potential coulometric reduction at the platinum electrode, appeared to be feasible. The

coulometric reduction was slow, taking about 45 minutes in a volume of 10 ml, compared with the more usual 20 to 25 minutes for a system such as Fe^{3+} - Fe^{2+}. However, the fusion and dissolution were quite rapid and, as no valency adjustment or separation was required, the over-all time for analysis was reasonable (about 90 minutes).

In an attempt to decrease the time required for the coulometric reduction, the possibility of using the Meites[6] end-point procedure was investigated. This procedure is dependent upon the electrolysis current obeying an equation of the form

$$i_t = i_0 c^{-Kt}$$

where i_0 is the initial current and i_t the current after time t. It was found that a current - time plot of the reduction of ruthenium showed two distinct rates of reaction, the first rapid and complete in less than 10 minutes and corresponding to about 50 per cent. of the titre, the second slow and complete only after about 45 minutes. With such a system the Meites end-point technique cannot be applied to the first slope, which is curved, and there appeared to be little advantage to be gained from applying the technique to the second slope. It is interesting, however, to compare this finding with that of Stenina and Agasyan,[3] who postulate an intermediate binuclear compound of ruthenium(IV) and ruthenium(III) during the constant-current coulometric reduction of ruthenium(IV) with the electrogenerated Ti^{3+}.

Quantitative determinations of ruthenium in ruthenium metal, uranium - ruthenium alloy and uranium - ruthenium carbide—The E_0' value found for the reduction of quadrivalent ruthenium as the $[Ru_2O]^{6+}$ chlorocomplex indicates that it should be possible to achieve 99·9 per cent. reduction at an applied potential of +0·185 V *versus* the S.C.E. This was attempted with 3-mg amounts of ruthenium, prepared as described above, in 5 M hydrochloric acid. It was found that at this potential the electrolysis current remained in the region of 50 to 100 μA for a considerable time, and that the time required to reduce the final 1 to 2 per cent. of ruthenium(IV) was unacceptably long. The rate of electrolysis could be increased and background currents of less than 10 μA could be achieved if the applied potential was decreased to +0·05 V *versus* the S.C.E. At this potential, electrolysis times of 30 minutes were achieved and spectrophotometric examination of the reduced solution showed no trace of quadrivalent ruthenium. This over-potential, which is typical of reactions of irreversible behaviour, was used in all subsequent work. Blank values determined on aliquots of solution prepared in exactly the same way as the ruthenium solution were found to be about 1 per cent. of the titre for a determination based upon 3 mg of ruthenium. No evidence was found for the presence of kinetic or induced blanks.

METHOD

DISSOLUTION OF RUTHENIUM-CONTAINING SAMPLES—

Crush and grind the sample to pass through a 100-mesh sieve. Weigh a portion containing not more than 100 mg of ruthenium and add it to 0·5 g of sodium peroxide contained in a 15-ml recrystallised alumina crucible. Mix by rotating the crucible at an angle of 45°. Add 0·5 g of sodium hydroxide, in pellet form, to the contents of the crucible. Place the crucible in a muffle furnace at 600° C and cover with an alumina lid. Remove the crucible from the furnace after 15 minutes and allow it to cool. Add 1 ml of water to the melt and replace the lid for 1 minute. Add a further 0·5 ml of water and swirl the contents of the crucible. Warm gently and continue swirling the crucible until the melt is completely dispersed. Add the solution, dropwise, to 10 ml of 5 M hydrochloric acid in a 100-ml beaker. Rinse the crucible with two 1-ml portions of concentrated hydrochloric acid followed by two 1-ml portions of water and add the rinsings to the beaker. Warm the solution in 50 to 75 ml of 5 M hydrochloric acid on the hot-plate for about 10 minutes. Cool, and dilute to give 100 ml of solution 5 M in hydrochloric acid.

Controlled-potential coulometric determination of ruthenium—Take an aliquot of the solution prepared as described above containing 2 to 4 mg of ruthenium, and transfer it to the electrolysis cell. Clean the platinum electrode for every determination by boiling it in nitric acid, rinsing in distilled water and igniting in the flame of a Meker burner. Carry out pre-electrolysis at +0·6 V *versus* the S.C.E. until a background current of less than 10 μA is obtained. Adjust the applied potential to +0·05 V *versus* the S.C.E. and electrolyse until a background current of less than 10 μA is again produced. Carry out a blank determination with reagents processed in the same manner as for ruthenium-containing samples. Ensure that the volume of the blank aliquot is the same as for the ruthenium solution.

Calculate the weight of ruthenium as follows—

$$W = \frac{N \times 101{\cdot}07 \times F}{96{,}487 \times 10^{-3}}$$

where W is the weight of ruthenium, mg; N, the scaler and register readings; and F, the coulometer factor, C counts^{-1}.

Results

Results for the determination of ruthenium in Specpure ruthenium metal, uranium - ruthenium alloy and uranium - ruthenium carbide are given in Table I. Uranium would not be expected to interfere in the determination of ruthenium at an applied potential of +0·05 V and this is confirmed in Table I. Uranium cannot, however, be determined by controlled-potential coulometry in the presence of ruthenium because of the reduction of ruthenium(III) at the mercury pool at the potential necessary for the reduction of UO_2^{2+} to U^{4+}. This interference was avoided by heating aliquots of the solution with perchloric and sulphuric acids to remove ruthenium by volatilisation prior to the determination of uranium by controlled-potential coulometry.

TABLE I

DETERMINATION OF RUTHENIUM IN RUTHENIUM METAL, URANIUM - RUTHENIUM ALLOY AND URANIUM - RUTHENIUM CARBIDE

Sample	Recovery, per cent.	No of determinations	Coefficient of variation, per cent.
Specpure ruthenium	Ru 99·8	8	1·0
Uranium - ruthenium alloy ..	Ru 50·7	6	1·1
	U 49·4		
	100·1		
Uranium - ruthenium carbide ..	Ru 29·5	6	1·0
	U 68·3		
	C 2·3		
	100·1		

Discussion

The analytical chemistry of ruthenium is complicated not only by the various valency states but also by the variety of complexes possible for each valency state.[1,4] Spectrophotometric methods for the determination of ruthenium in chloride solution usually overcome these difficulties by reducing the sample solution to ruthenium(III) and then re-oxidising to a ruthenium(IV) chlorocomplex with an absorbance peak at 485 nm.[5] It has been shown, by a combination of controlled-potential coulometry and spectrophotometry, that dissolution of ruthenium metal and various ruthenium compounds in alkaline peroxide medium followed by extraction into hydrochloric acid gives a reproducible ruthenium(IV) complex. Subsequent adjustment of the valency of the ruthenium is unnecessary, and the ruthenium can be determined directly by a controlled-potential coulometric reduction at +0·05 V *versus* the S.C.E. Further electrolytic reduction of ruthenium(III) is readily achieved in chloride medium to give blue chlorocomplexes of ruthenium(II), but this process is not suitable for the analysis, probably because of a catalytic reaction in which hydrogen ions are reduced. A reversible Ru^{4+} - Ru^{3+} couple at +0·805 V *versus* the S.C.E. can also be used for the controlled-potential coulometric determination of ruthenium, but this Ru^{4+} complex is not obtained by the method of dissolution described. This latter redox couple has been previously reported in a method for the potentiostatic coulometry of ruthenium.[3]

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Pre-concentration of Carbonyl Compounds from their Medium Followed by Polarographic Determination of their Azomethine Derivatives

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A method for the pre-concentration of carbonyl compounds on a chromatographic column via the formation of an azomethine derivative is described. The subsequent elution of the derivative is monitored polarographically.

The pre-concentration of carbonyl compounds from their medium is important in many fields, especially in food flavourings. Polarographic techniques are suitable for the determinations of this class of compound, and the optimum conditions for determining various types of carbonyl compound have already been reported.[1,2,3,4]

In the course of a study on the chromato-polarographic determination of carbonyl compounds a method has been developed for the pre-concentration of lower aliphatic carbonyl compounds and their separation from aromatic aldehydes and ketones.

Parsons[5] has studied the rate of formation of 2,4-dinitrophenylhydrazones on a Celite column impregnated with the reagent. The derivative is eluted from the column with benzene and the reaction followed spectrophotometrically. For the analysis of the carbonyl content of butter fat and butter-fat volatiles, a good separation into classes, *i.e.*, alkanals, alk-2-enals and alk-2,4-dienals, was achieved for the volatile constituents, but the carbonyl content of the involatiles was too high to allow a complete separation.

The present study has been based on the formation of the semicarbazone derivatives as in this instance the reagent itself is electro-inactive, whereas with 2,4-dinitrophenylhydrazine a well defined wave is obtained by reduction of the nitro-group. The optimum conditions for the formation and polarographic determination of semicarbazones has been established.[1,2] The reduction of the azomethine group occurs via a 4-electron process to form the primary amine and urea.

$$R{-}\underbrace{CH{=}N{-}NH{-}\overset{\overset{O}{\|}}{C}{-}NH_2}_{H^+} + 4e^- + 3H^+ \rightarrow R{-}CH_2{-}NH_2 + H_2N{-}\overset{\overset{O}{\|}}{C}{-}NH_2$$

As the reduction occurs through the protonated complex the polarographic wave shows a conventional dependence on pH with the limiting value of the current extending over the pH range 2 to 4·5. Below pH 2 the hydrolysis of the semicarbazones of aliphatic carbonyls is rapid; above pH 4·5 the rate of protonation becomes the limiting step. It is possible to form the derivative *in situ* by adding the carbonyl compound to a buffered supporting electrolyte containing a large excess of the reagent. The use of a buffered medium for this reaction has an important advantage; the rate of formation of semicarbazones shows a peak-shaped dependence on pH with a maximum at about pH 4·5.[6] As this pH also corresponds to the limiting current region for the reduction, an acetate buffer (containing 50 per cent. of ethanol) can conveniently be used.

Although a recent study[4] has shown that the Girard T derivatives of aliphatic aldehydes give better defined reduction waves than the corresponding semicarbazones, their formation and subsequent chromatographic separation are far less favourable.

Experimental

Reagents—

Carbonyl compounds were obtained commercially. Standard solutions (10^{-2} M) in analytical-reagent grade ethanol were prepared. Unless otherwise stated all reagents were of analytical-reagent grade.

Buffer solution, 0·2 M sodium acetate - 2·0 M acetic acid.

Apparatus—

Polarograms were recorded on a Radelkis polarograph type OH102 (Metrimpex, Hungary). The polarographic vessel was a Kalousek cell with a separated reference electrode (saturated calomel electrode).

The capillary used had the following characteristics: outflow velocity m = 2·04 mg s^{-1} and drop time t = 4·1 s, at the potential of the S.C.E. mercury pressure h = 60 cm.

Column preparation—

Two columns were used, the first a short Celite column (B.D.H., 30 to 80 mesh) impregnated with the reagent for the formation and concentration of the derivative, followed by the main chromatographic column for the separation. Silica gel (B.D.H., 60 to 120 mesh) was used for this column without any pre-treatment.

Reaction column—Fifty grams of Celite were impregnated with a solution of 0·45 g of semicarbazide hydrochloride in 2 ml of 85 per cent. orthophosphoric acid (sp.gr. 1·75) diluted with 8 ml of water. This was transferred to a column (2 cm in diameter) fitted with a sintered-glass plug, and washed with 50 ml of absolute ethanol. This gave a reaction layer about 10 cm in length. A slight loss of reagent occurs during the washing process. This column is capable of retaining carbonyl compounds from samples of up to 100 ml total volume in ethanol containing 10 per cent. of water. For smaller samples, *e.g.*, 10 ml, a 2-cm reaction layer is sufficient. In the latter instance 10 g of Celite are adequate.

Separation column—The second column (1 cm in diameter) contained sufficient silica gel to form a layer 10 cm in length. The reaction column was fitted into the top of the separation column, after the formation of the semicarbazones.

Procedure—

The sample, containing between 0·1 and 1 μmoles of the carbonyl compound in up to 100 ml of ethanol containing 10 per cent. of water, is slowly percolated through the reaction column. The semicarbazone derivatives are formed on the reaction column. The semicarbazones are eluted with either absolute ethanol or ethyl acetate - ethanol (1 + 1). Aliquots of the eluent selected from retention data were diluted with an equal volume of the aqueous acetate buffer and the polarogram measured between −0·5 and −1·7 V *versus* S.C.E.

Results and discussion

Several solvent systems were examined with the aim of separating the semicarbazones of the lower aliphatic aldehydes and ketones. The range of solvents, however, was limited in that the polarographic reaction wave was only well defined in polar solvents. Hence, various mixtures of ethanol, dimethylformamide and ethyl acetate were chosen for study. The semicarbazones of acetone and acetaldehyde were chosen as representative of the lower aliphatic carbonyls and benzaldehyde and several substituted benzaldehyde semicarbazones for the aromatic series.

All of the solvent systems examined gave incomplete separation of the semicarbazone derivatives of acetone and acetaldehyde. However, ethyl acetate - ethanol (1 + 1) was found to be a good solvent for the separation of the aromatic semicarbazones from the aliphatic fraction. Both acetone and acetaldehyde semicarbazones were sparingly soluble in this solvent and thus remained at the top of the silica gel column. After elution of aromatic derivatives the column was washed with ethanol alone when the acetone and acetaldehyde semicarbazones were eluted quantitatively within the first 10 ml.

It was possible to achieve a reasonable degree of separation of benzaldehyde semicarbazone and the 4-methoxy and 4-chloro derivatives with the ethanol - ethyl acetate

solvent system. Measurement of successive 2-ml aliquots of the eluate showed distinctive peaks as the components emerged, but there was some overlapping. Although it would almost certainly be possible to improve this separation, *e.g.*, by increasing the length of the column or modifying the eluting solvent, this was not studied because of the doubtful usefulness of this particular separation. A summary of the optimum conditions for the separation of certain mixtures of carbonyl compounds is given in Table I.

TABLE I

OPTIMUM CONDITIONS FOR THE SEPARATION OF MIXTURES OF CARBONYL COMPOUNDS AFTER FORMATION OF THEIR SEMICARBAZONE DERIVATIVES

Separation column 10 cm, flow-rate 1 ml minute^{-1}

Carbonyl compound	Carbonyl added, μmoles	Carbonyl recovered, μmoles	Recovery, per cent.	Eluting solvent	Separation	Retention volume, ml
Acetone	0·25	0·20	80 ± 2	Ethanol	Complete*	5
4-Chlorobenzaldehyde	0·5	0·49	98 ± 2	Ethanol - ethyl acetate		7
Acetone	0·25	0·20	80 ± 2	Ethanol or Ethanol - ethyl acetate - dimethylformamide	None†	Both compounds approximately 18
Acetaldehyde	0·25	0·20	80 ± 2			
Benzaldehyde	0·50	—	—	Ethyl acetate - ethanol	Partial‡	10
4-Chlorobenzaldehyde	0·50	—	—			8
4-Methoxybenzaldehyde	0·50	—	—			6

* 4-Chlorobenzaldehyde derivative detected in 5 to 10-ml fraction.
Acetone semicarbazone eluted with ethanol, after 20 ml of ethanol - ethyl acetate had been run through the column.

† The polarograph waves were ill defined in the ethanol - ethyl acetate - dimethylformamide solvent mixture.

‡ The semicarbazones would appear to exist as broad drawn-out bands on the column. Consequently some degree of overlap was observed.

It is also possible to use a flow-through polarographic cell[7] to monitor the eluate.

Although the present work was unsuccessful in developing a chromato-polarographic method for the separation of aliphatic carbonyl semicarbazone derivatives, it was felt that the development of a technique for pre-concentration of aldehyde and ketone semicarbazones from their medium and their separation into broad classes is of analytical importance. An important consideration here is the fact that a wide range of aldehyde and ketone semicarbazones have similar diffusion current constants,[2] hence it is possible to apply the above method for total carbonyl content.

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Some Observations on the Analytical Usefulness of Electrochemiluminescence for the Determination of Microgram Amounts of Aromatic Hydrocarbons*

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Measurement of the electrogenerated chemiluminescence of twenty-four aromatic compounds in dimethylformamide solution has been investigated as a potential analytical technique for their determination. The emission spectra, calibration results and limits of determination are presented. Experimental requirements and optimum conditions for the electrogeneration of the light-emitting species are described.

Emission of radiation from solutions during electrolysis has been observed for solutions of metal salts,[1] Grignard reagents in ethers[2] and alkaline solutions of fluorescent[3] and chemiluminescent[4] compounds. The electrolysis of some aromatic hydrocarbons in solvents such as dimethylformamide and acetonitrile has recently been shown to result in emission of visible radiation.[5] This phenomenon is referred to as electrochemiluminescence (ECL), and the mechanism of the processes involved has been extensively studied by several workers.[6 to 16] With the exception of the study by Cruser and Bard,[17] and the application of electrochemiluminescence measurement to the determination of polycyclic aromatic hydrocarbons in liver lipids by Bobr, Kozlov and Mikhailovskii,[18] few results are available concerning the potential and range of application of ECL to the identification and determination of microgram amounts of organic compounds. In a previous short communication[19] we have reported some initial observations on the analytical application of the technique of ECL measurement. This paper describes the apparatus developed for the study of the parameters governing ECL emission, and reports the ECL characteristics of a range of aromatic hydrocarbons and heterocyclic compounds.

Experimental

Apparatus—

A diagrammatic representation of the apparatus used is shown in Figs. 1 and 2. The wave form is applied to the working electrode of the cell by a function generator (Hewlett-

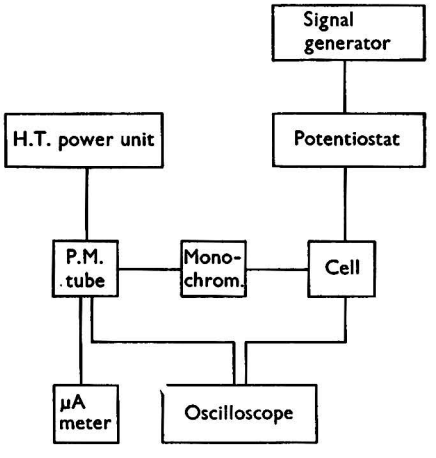

Fig. 1. Diagrammatic representation of apparatus used

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Packard Corporation, HP3300A) two-channel triangular, square or sine-wave, variable frequency (d.c. to 100-kHz with single sweep and phase-lock facilities) and operational amplifier potentiostat with Philbrick PF85AU amplifiers and Philbrick P66A booster. The potentiostat circuit is as described by Schwarz and Shain[20] with a differential input to single-ended output follower and provides up to 100-mA signal.

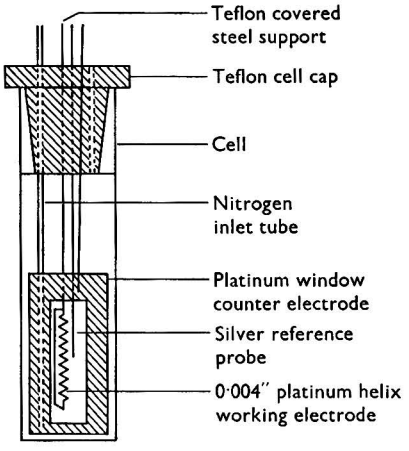

Fig. 2. Electrochemiluminescence cell assembly

The ECL sample cell is a fused silica spectrofluorimeter cell (10 × 10 × 40 cm) with a machined Teflon stopper into which the three electrodes and nitrogen inlet and outlet tubes are sealed. The working electrode is a platinum helix supported on a steel rod coated with PTFE. This rod acts as electrical connection to the potentiostat. The counter electrode is a platinum sheet spot-welded to a platinum-rod contact. The reference electrode is a silver wire in the same cell. Although the silver electrode under these conditions is only a pseudo-reference electrode, it exhibits a potential that remains constant during the course of an experiment and that can be related to the potential of the S.C.E. (−0·33 V).

The radiation emitted from the working electrode is viewed through the counter electrode by an 11-stage photomultiplier tube (EMI9601B) via a metal dielectric interference monochromator (Barr and Stroud Ltd., London). The 3-mm slit of this monochromator results in a spectral half-band width of about 25 nm, over a spectral range from 400 to 700 nm. The photomultiplier photocurrent is displayed on an ultra-sensitive micro ammeter (RCA Type WV84C), oscilloscope (Hewlett-Packard 175A), or pen recorder (Servoscribe Type AE511, Smiths Industries).

REAGENTS—

Dimethylformamide (British Drug Houses Ltd. laboratory-reagent grade) was used as solvent throughout. It was dried over anhydrous potassium carbonate and then over a molecular sieve (Lind 5A). The dried solvent was then distilled under vacuum in a fractional distillation assembly. The distillate was stored in brown-glass reagent bottles until required.

Nitrogen—Oxygen-free nitrogen (British Oxygen Co., oxygen-free grade) was freed from traces of oxygen by passage through a 2 per cent. solution of the sodium salt of anthraquinone-*p*-sulphonic acid in 2 N sodium hydroxide containing granulated zinc. The nitrogen was then dried by passage through concentrated sulphuric acid and magnesium perchlorate. The gas was then finally passed through a Dreschel bottle containing dimethylformamide to ensure saturation of the gas with solvent and minimise evaporation of solvent in the sample cell during the de-gassing procedure.

Electrolyte—Tetrabutylammonium perchlorate (TBAP). This was prepared by neutralisation of tetrabutylammonium hydroxide solution (B.D.H., general-purpose reagent grade) with 70 per cent. analytical-reagent grade perchloric acid. The product was recrystallised twice from a water - ethanol mixture and dried under reduced pressure at 95° C for 24 hours.

Some of the organic compounds, the ECL characteristics of which were studied, were obtained commercially, while others were supplied as samples by courtesy of the British American Tobacco Company. The compounds were purified before use by vacuum sublimation or recrystallisation.

General experimental procedure

Emission spectra—

The emission spectra were measured for 10^{-3} M solutions of each compound in dimethylformamide containing 10^{-2} M TBAP, and the dissolved gases present were removed by alternate freezing and thawing cycles or by nitrogen purging. The solution was placed in the cell, and a 30-Hz square-wave signal of increasing amplitude was applied to the working electrode while the solution was gently stirred by nitrogen. When light emission was detected, the optimum frequency and wavelength of maximum emission were established, and the current - voltage (cyclic voltammogram) characteristics examined. The dependence of the light-emission intensity on the applied voltage was also investigated.

When no light emission was observed with a 30-Hz square wave, a low frequency (1 Hz) signal was applied to the electrode and the effect of stopping the stirring was investigated.

Spectra were obtained for the sample solutions in dimethylformamide and TBAP electrolyte (10^{-2} or 10^{-1} M, depending on which concentration produced the greater light intensity). The experimentally established optimum frequency and applied voltage were used. The light intensity of the emission was found to reach a stable value for most of the compounds investigated within 10 seconds (at 30 Hz) or 30 seconds (at 1 Hz). The spectrum was recorded manually, and the mean light intensity was measured at intervals of 20 nm between 400 and 700 nm.

The calibration data (ECL intensity at $\lambda_{max.}$ *versus* concentration) were obtained by preparing a range of dimethylformamide - TBAP solutions containing various sample concentrations, and recording the steady value of the light intensity obtained for each solution under optimum conditions. Alternatively, the light intensity obtained initially on application of the optimum voltage was recorded for each solution at the wavelength of maximum emission. In each instance the calibration graphs were plotted as $\log_{10}$ intensity *versus* $\log_{10}$ sample concentration.

Results and discussion

A wide range of compounds was examined for electrochemiluminescence in dimethylformamide solvent. In all instances both the emission spectra and the current - voltage graphs were recorded. The potentials at which the peak intensity of light emission occurred was compared with the current peaks on the current - voltage graph (cyclic voltammogram) corresponding to the formation of the radical anion and cations. Of the compounds studied, some twenty-four of those that showed intense emission were chosen for further study and their ECL spectra shown in Fig. 3. These spectra are uncorrected for the response characteristics of the EMI9601B photomultiplier and the monochromator used. In most instances broad-band spectra that resemble the fluorescence spectra of the compounds are obtained. For several compounds (*e.g.*, phenanthrene, 2,2′-binaphthyl), long wavelength emission not found in the corresponding fluorescence spectra occurs. Several compounds were shown to exhibit only weak ECL emission and were not examined further. *N*-Phenylpyrrole, *NN*′-di-2-naphthyl-*p*-phenylenediamine, *p*-methoxybenzoylnaphthalene and acetyl-1-naphthylamine exhibit weak emission in this way. Compounds that were found not to exhibit ECL emission in dimethylformamide under the conditions used in this study include diphenylbenzidine, 2,2′-dihydroxybiphenyl, 2,2′-dimethoxybiphenyl, 1,10-phenanthroline, acridine, benzanthrone, 1-naphthoic acid, 4-phenylazophenol, quinaldine, naphthalene-1,5-diol, 2-aminothiazole, 3-methylisoquinoline, azulene and various hydroxynaphthalenesulphonic acids.

For the twenty-four compounds examined in detail, the results obtained are shown in Table I. Four distinct types of electrochemiluminescent behaviour were observed experimentally for the compounds under the experimental conditions used. The type of behaviour assigned to each compound in Table I is responsible for the principal emission of analytical use. Most compounds also show one or more of the other types of behaviour at much lower light-emission intensities.

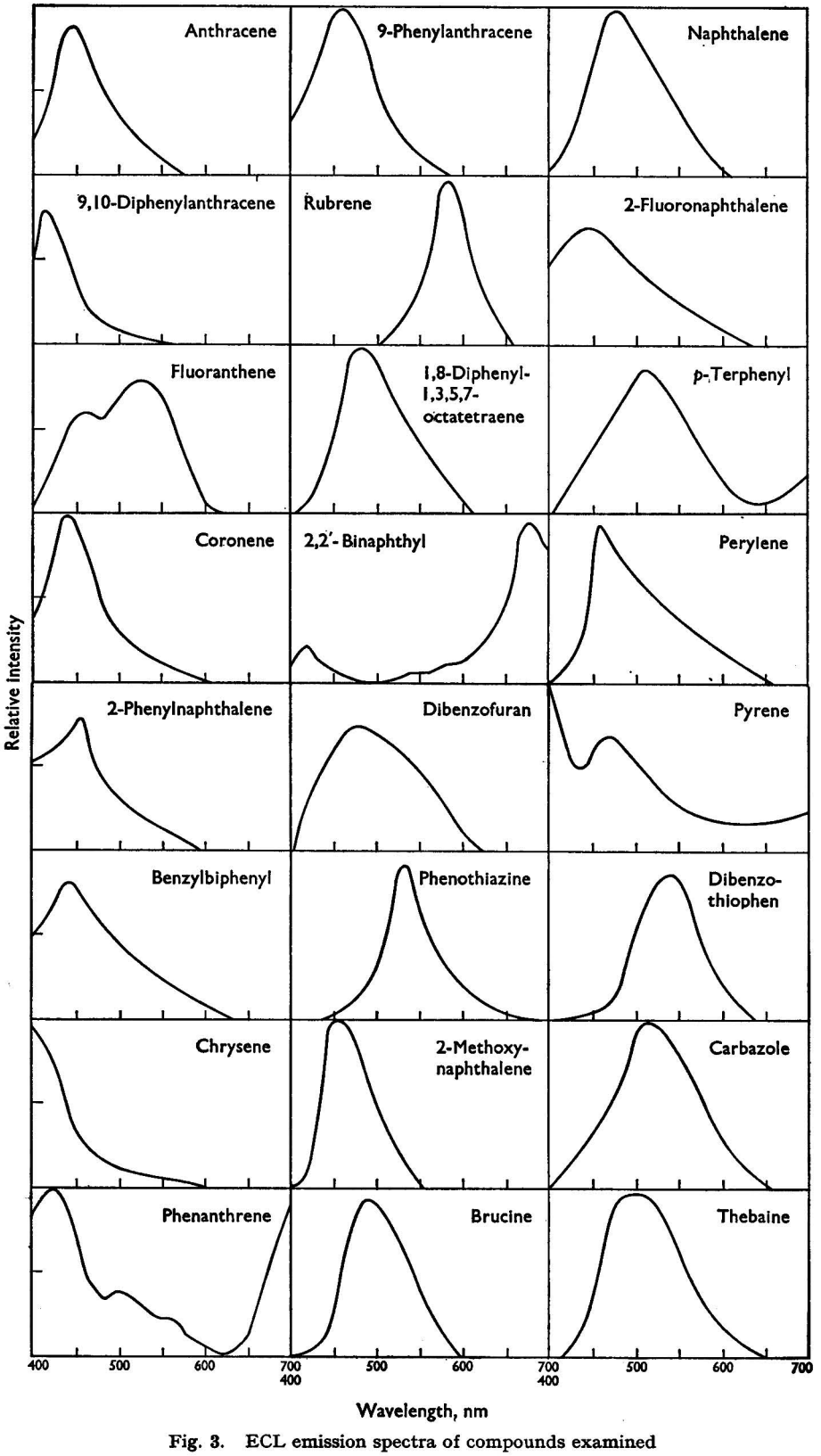

Fig. 3. ECL emission spectra of compounds examined

TABLE I

RESULTS

Compound	Normal ECL max., nm	Fluorescence emission max., nm[21,22]	Range for calibration graph, M	Type of emission (see text)	Optimum frequency, Hz	Preferred waveform
Coronene	440	417, 440	$10^{-3} \rightarrow 10^{-6}$	a	30	Square wave
Rubrene	575	575	$10^{-3} \rightarrow 10^{-7}$	a	30	Square wave
Anthracene	450	375, 395, 419, 444, 474	$10^{-3} \rightarrow 5 \cdot 10^{-7}$	a	10	Square wave
9-Phenylanthracene	430	430	$10^{-3} \rightarrow 10^{-7}$	a	30	Square wave
9,10-Diphenyl-anthracene	420	430	$10^{-3} \rightarrow 10^{-7}$	a	50	Square wave
Pyrene	400, 480	410, 460, 397, 385	$10^{-3} \rightarrow 5 \cdot 10^{-5}$	a	5	Square wave
Fluoranthene	460, 525	438, 462, 482	$10^{-3} \rightarrow 2 \cdot 10^{-5}$	a	30	Square wave
Chrysene	—	387, 400, 408, 423, 450	$10^{-3} \rightarrow 2 \cdot 10^{-5}$	d	20	Square wave
1,8-Diphenyl-1,3,5,7-octatetraene	480	—	$10^{-3} \rightarrow 2 \cdot 10^{-5}$	a	10	Square wave
Phenanthrene	520,[1] 410[2]	398, 420, 448	$5 \cdot 10^{-3} \rightarrow 2 \cdot 10^{-5}$	a[2], d[1]	50	Square wave
Perylene	455	442, 462, 494, 520	—	a	10	Square wave
Binaphthyl	420, 670	385	$2 \cdot 10^{-3} \rightarrow 2 \cdot 10^{-5}$	a	40	Square wave
Benzylbiphenyl	440	330, 350	—	c	1	Square wave
p-Terphenyl	510	360	—	d	30	Square wave
Naphthalene	470	320	—	d	30	Square wave
Dibenzofuran	475	410, 440, 470	—	a	30	Square wave
Carbazole	570	408, 429	—	d	30	Square wave
Phenothiazine	530	—	$5 \cdot 10^{-3} \rightarrow 10^{-5}$	b	0·1	$-2 \rightarrow -3$ Triangular wave
Dibenzothiophen	540	—	—	b	1	$-0 \cdot 5 \rightarrow -3 \cdot 5$ Square wave
2-Methoxynaphthalene	450	—	only above 10^{-3}	c + d	d.c.	−2·95 (stirred)
Brucine	490	—	only above 10^{-3}	c	0·1	$-2 \rightarrow -4$ Triangular wave
Thebaine	485	—	only above 10^{-3}	c	10	Square wave
2-Fluoronaphthalene	450	—	only above 10^{-3}	d	30	Square wave
2-Phenylnaphthalene	445	—	only above 10^{-3}	a	50	Square wave

The four types of behaviour are as follows.

(a) Radical cation - anion annihilation. This appears to be the most common type of mechanism involved in ECL emission for aromatic hydrocarbons where the energy available from the cation - anion radical reaction $R^+ + R^- \rightarrow R^* + R$ is sufficient to form the excited singlet state from which ECL emission can occur. This type of process is recognisable from the necessity that emission only occurs after a complete voltage sweep from negative to positive potentials corresponding to the formation of the anion and cation-free radicals. This type of process results in ECL, for example, for rubrene, fluoranthene and diphenylanthracene, when the possibility of forming reasonably stable free radicals exists. The introduction of functional groups precludes the emission by this process as the radical ions, particularly the cation, are no longer stable.

(b) When the radical cation or anion reacts with some impurity species, *e.g.*, traces of molecular oxygen or water. This type of emission can be recognised experimentally when the electrode is held at the potential required to generate either R^+ or R^-, rather than when a square-wave form is applied. A steady emission of light may then occur as the radicals formed diffuse into the bulk of the solution and react with the impurities. We have observed behaviour of this type for the compounds phenothiazine and dibenzophenothiophene.

(c) When the light emission is only observed on repeated cycling of the potential. Clearly in this instance the products of the primary electrode reaction are responsible for the light emission as no light is observed on the initial scan. This type of emission is usually observed as a band at longer wavelengths than ECL emission by other processes. The emission intensity increases with time, but usually remains weaker than the ECL stimulated by the primary

process. This type of emission has been observed, for example, for chrysene and 2-fluoronaphthalene. For some compounds, however, it appears to be the principal mode of emission, *e.g.*, with the alkaloids brucine and thebaine.

(d) When light emission occurs at potentials beyond those at which normal solution electrolyte reactions occur, *e.g.*, with carbazole and *p*-terphenyl. We are currently undertaking a study of this type of behaviour to elucidate the mechanism of the process or processes involved, but it seems probable that higher reduced or oxidised species, for example the dianion or even products resulting from the reduction or oxidation of the solvent or supporting electrolyte, are involved.

CALIBRATION DATA AND LIMITS OF DETERMINATION—

Typical calibration graphs prepared by the procedure described are shown in Fig. 4 for twelve of the compounds examined. It will be observed that when $\log_{10}$ intensity is plotted *versus* $\log_{10}$ concentration, linear calibrations are obtained. Deviations from linearity are observed in several instances in the higher concentration ranges and can be ascribed to concentration quenching. The dynamic range of the technique is good, as many of the calibration graphs are linear over a 1000-fold range of concentrations.

ECL emission was observed for brucine, thebaine, 2-methoxynaphthalene, 2-fluoronaphthalene and 2-phenylnaphthalene only at concentrations greater than 10^{-3} M. The lower concentration limit of the calibration graph for each compound represents the practical limit of determination with our apparatus. The absolute determination limits obtainable reflect the efficiency of the particular experimental assembly used, and can be improved by reduction

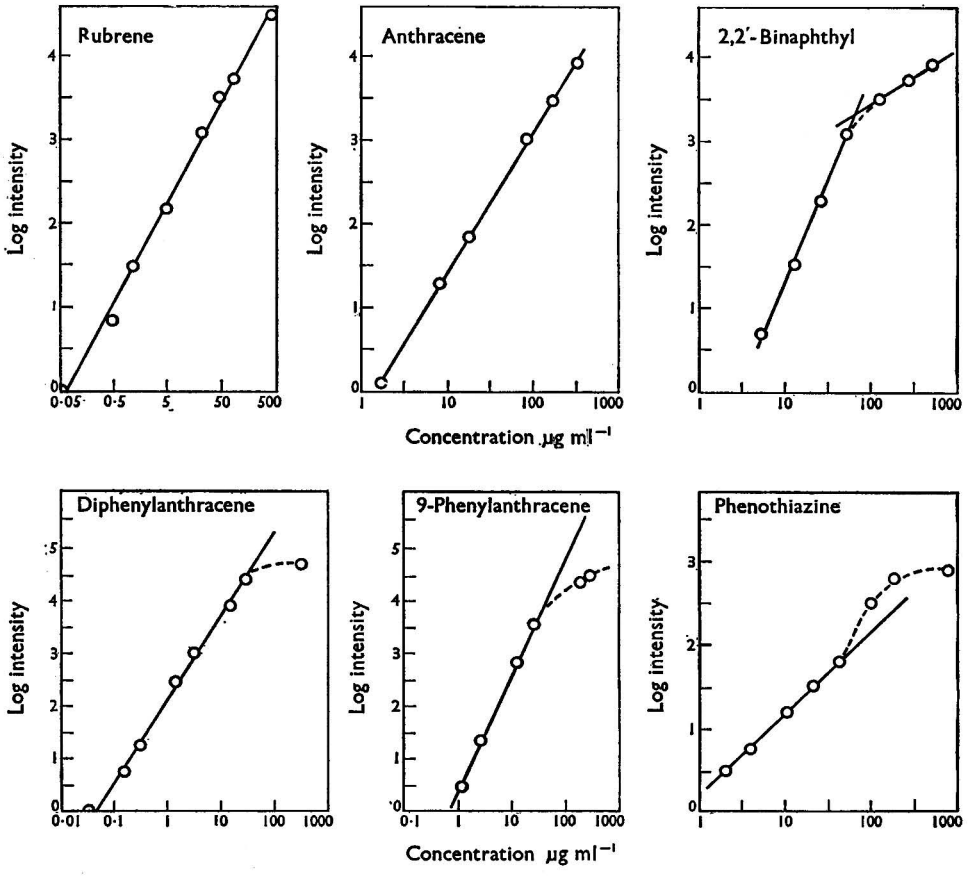

Fig. 4. Calibration graphs for ECL emission *versus* concentration of compounds in DMF

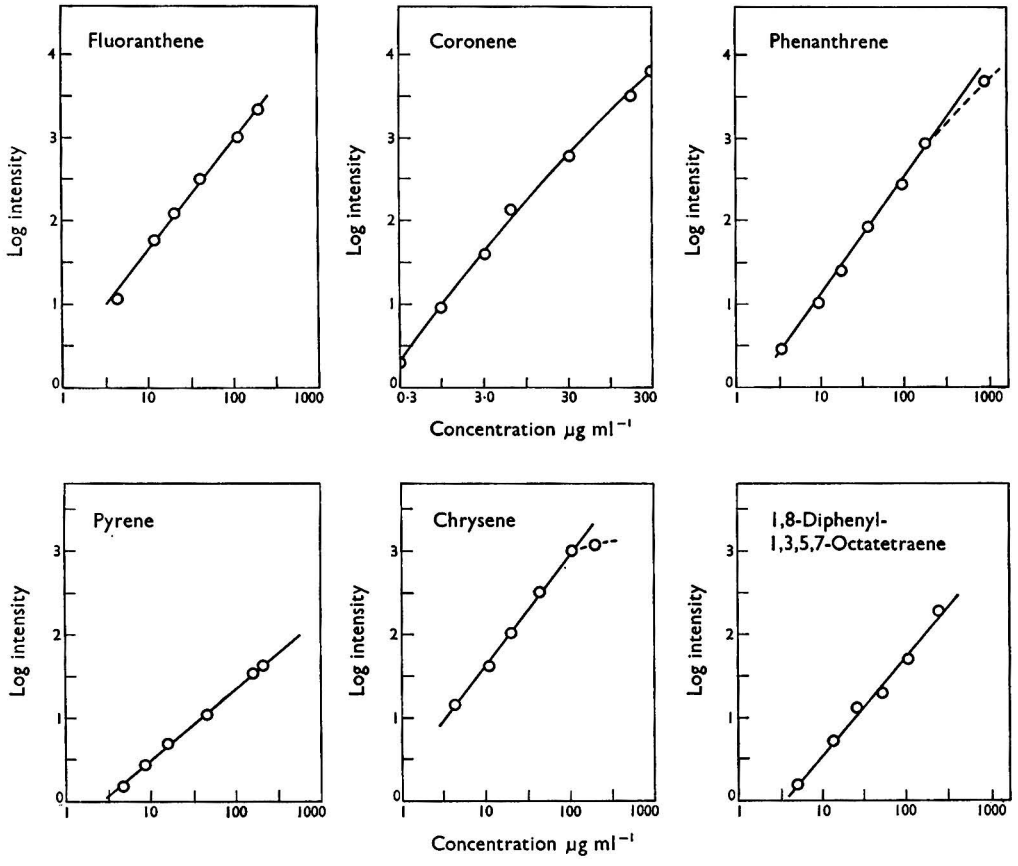

Fig. 4 (*contd.*) Calibration graphs for ECL emission *versus* concentration of compounds in DMF

in the sample volume required, careful monochromator design and selection of photomultiplier and electronic components.

It was observed that deviations in linearity in concentration - light intensity relationships were often observed in those instances in which simple cation - anion annihilation was not operable, *i.e.*, those observed to fit into categories (b), (c) and (d). This at once imposes a limitation on the technique as only the relatively simple polycyclic hydrocarbons, in which the possibility of forming a relatively stable free radical exists, show this type of process. The introduction of functional groupings such as hydroxyl and amino precludes this, as noted previously. On the other hand, this limitation provides a degree of selectivity over the conventional fluorescence technique. It would seem, therefore, that the wide range of "energy deficient" electrochemiluminescent processes, where the energy of interaction of cation and anion radicals is insufficient to form the singlet state, are not amenable to quantitative ECL determination or at least can only be determined over a fairly limited concentration range. It is possible, however, that the removal of the randomness of the quenching process, *i.e.*, by the addition of a specific anion or cation quencher to form a radical at a potential such that interaction will lead to the triplet state, may provide the solution. An advantage of ECL over conventional spectrofluorimetry is that as there is no source of excitation no problems associated with scattered radiation arise. It might also be possible, by controlling the electrolysis potential, to determine selectively individual components of simple mixtures whose fluorescence excitation and emission spectra are similar. Work on the analysis of mixtures of this type is currently in progress.

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Voltammetric Studies with Different Electrode Systems

Part IV.* Determination of Silver by Using a Silver - Molybdenum System

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Silver is determined at trace level (1 to 100 p.p.m.) in organic solvents and alkalis by using a semi-micro cell incorporating a silver - molybdenum system. The determination is carried out on a differential cathode-ray polarograph. With this electrode system only silver and mercury cations were reduced and the peak potentials were well separated. No chemical separations are involved. Factors affecting the silver reduction in organic solvents are discussed.

Silver is one of the impurities that influence photo-conductive effects in semi-conductors.[1] A method for its determination in some organic solvents of high purity, notably methanol and acetone, which may be used in the preparation of semi-conductor materials, is therefore required.

Polarographic reduction of silver and mercury occur together in almost all media and the separation of the two waves becomes very difficult, especially when a dropping-mercury electrode is used, as the dissolution wave of mercury is always obtained.

The silver indicator electrode has been thoroughly examined in potentiometry,[2,3] in which it is responsive mainly to silver and mercury ions. Silver used as the cathode in polarography may overcome the difficulties encountered with the dropping-mercury electrode, but it failed to give rise to reproducible curves on a d.c. polarograph, and an investigation was carried out by using a differential cathode-ray polarograph.

The choice of reference electrode also presents difficulty in the polarography of silver ions, as neither a saturated calomel nor a mercury-pool electrode can be used. A molybdenum-wire electrode, which has been used as a reference electrode under diverse experimental conditions[4,5,6,7,8] is primarily a pH electrode,[9,10] but while the pH remains constant, it functions as a reference electrode in polarography and potentiometry.[11] In a polarographic study of silver reduction, the dropping-mercury electrode - molybdenum system has been used with partial success.[12] The molybdenum reference electrode was, therefore, used in silver voltammetry.

The silver - molybdenum bi-metallic system has been studied in great detail,[13,14] and it was shown that of all of the cations only mercury and silver reduction curves were obtained; an S-shaped mercury wave was obtained at −0·40 V and a silver peak at −0·60 V, thus eliminating the possibilities of interference. Moreover, with this system, no dissolution wave of mercury was obtained as with the dropping-mercury electrode.

With the bi-metallic system the construction of a semi-micro cell is facilitated and it can be used in mixed and non-aqueous solvents, unlike the dropping mercury - S.C.E. system, which gives irreproducible results because of irregular drop time.

Experimental

Apparatus—

A differential cathode-ray polarograph, Model A, 1660 (Southern Analytical Ltd.) was used, at 30° ± 0·1° C.

* For details of earlier parts of this series, see reference list, p. 859.

Semi-micro cell—A cell of 0·5-ml capacity was constructed by simply arranging silver (28 s.w.g.) and molybdenum (22 s.w.g.) wire electrodes in a cork that was inserted into a Pyrex glass tube of 1 cm diameter. An improved version, with facilities for inlet and outlet of nitrogen, as well as for inserting the burette tip, is shown in Fig. 1. The silver-wire electrode was prepared by sealing the wire in soft glass tubing. This electrode was cleaned with nitric acid (1 + 4) and the molybdenum electrode by rubbing with emery paper.

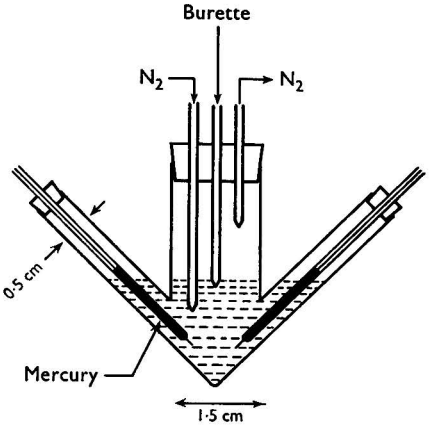

Fig. 1. Improved semi-micro cell: anode, molybdenum 4 mm; and cathode, silver 3 mm

REAGENTS—

Stock silver nitrate solution, 0·1 M.

Stock sulphosalicylic acid solution, 1 M.

Analytical-reagent grade solvents were used and were further purified by re-distilling three times.

RESULTS AND DISCUSSION

SILVER REDUCTION IN AQUEOUS SOLUTIONS—

In the semi-micro cell, the length of both electrodes is reduced to one fifth of that of normal electrodes. In view of the possibility of mutual polarisation in the bi-metallic electrode system, the silver reduction was re-examined for the small electrodes.

The curves obtained for the 0 and 100 slope component factors are shown in Fig. 2; this factor does not greatly affect the shape of the curve.

Current - concentration linearity is observed for the range 0·25 to 1·00 mM silver concentration, as shown in graph (*a*), Fig. 3. The peak potentials obtained vary between —0·55 and —0·60 V, being almost identical with those obtained with normal electrodes. It was also confirmed that no ions other than mercury(I) were reduced. The mercury(I) reduction wave intermingles with the residual oxygen wave starting from —0·2 V and does not interfere in the silver determination.

SILVER REDUCTION IN 50 PER CENT. METHANOL—

Current - concentration linearity was studied in 50 per cent. methanol, in which the solubility of the supporting electrolyte and the resistance of the solution are not affected as they are with the aqueous solution. Also in a pure non-aqueous solvent, the electrode behaviour becomes erratic. The current - concentration graph is a straight line over the range 0·25 to 1·00 mM silver concentration, as shown in Fig. 3 (*b*). Even in 50 per cent. organic solvents, the peak currents are much smaller than those with the aqueous solution for 1·00 mM silver concentration (2·0 μA compared with 6·30 μA in the aqueous solution).

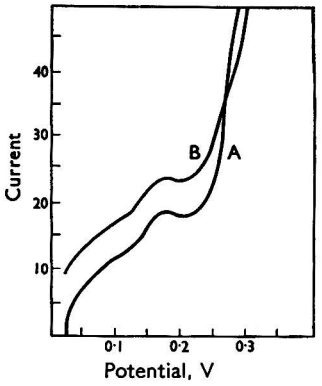

Fig. 2. Silver peak on silver (3 mm) cathode: anode, molybdenum 4 mm; silver concentration, 0·5 mM; shunt scale factor, 1·5 × 10; amplification factor, 1 × 100; and start potential, 0·4 V. Curves A and B were taken for 0 and 100 slope component, respectively; i_p (μA) = 2 × 10^{-4} × number of divisions × shunt scale factor × amplification factor; and E_p = −0·6 V for both curves: current in arbitrary units

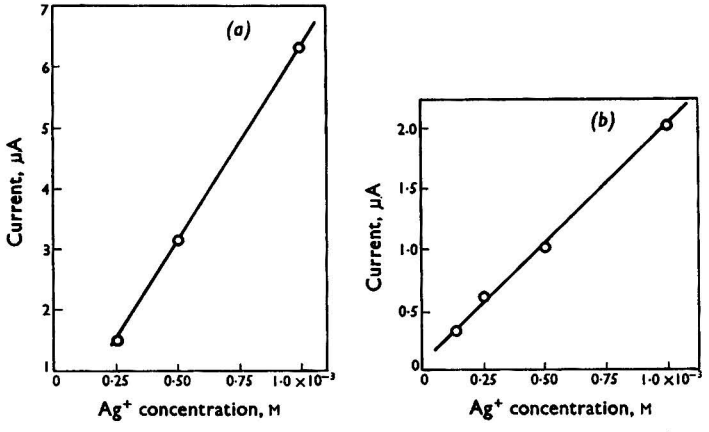

Fig. 3. Current - concentration graphs for Ag^+ ions: (*a*), in aqueous solution; and (*b*), in 50 per cent. methanol: semi-micro cell

Moreover, at lower silver concentrations, the peaks are not well defined, as shown in Fig. 4. The peak potentials do not vary much compared with those for the aqueous solution, E_p being −0·51 to −0·54 V. These results are in agreement with those obtained by Lietzke and Stoughton,[15] who reported that in 50 per cent. methanol the currents resulting from inorganic cation reductions were reduced but that the potentials were not much affected.

Similar reductions in peak currents were also obtained with aqueous mixtures of other organic solvents.

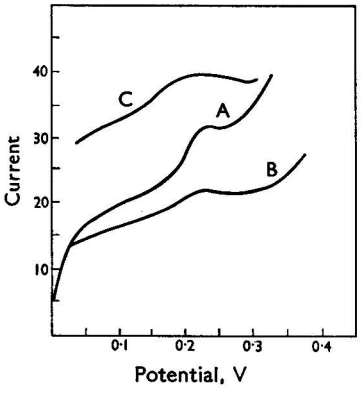

Fig. 4. Silver peaks for different concentrations in 50 per cent. methanol: cathode, silver 3 mm; anode, molybdenum 4 mm; for curves A and B, shunt scale factor and amplification factor 1 × 10 and 1 × 100, respectively, and for curve C, 6 × 1 and 1 × 100, respectively. Curves A, B and C were taken for 1·0, 1·5 and 0·25 mM silver concentration, respectively; start potential, 0·3 V for all of the curves; and E_p, −0·54, −0·53 and −0·51 V for curves A, B and C: current in arbitrary units

Another important drawback arising from the use of mixed solvents is the persistent oxygen wave, which is difficult to eliminate and especially so when dioxan is used. As the peak currents and potentials become progressively unsteady with increasing percentage of organic solvent, it is not possible to use larger amounts of the solvent sample. For these reasons, it is desirable not to carry out the silver determination in mixed solvents.

SYNTHETIC SAMPLES—

The difficulty mentioned above can be avoided by using a larger amount of the solvent and then removing it by evaporation. In the solvents used, *viz.*, methanol, acetone and dioxan, silver nitrate is very soluble,[16] and therefore the recoveries could readily be tested.

TABLE I

SILVER PEAK CURRENT AND POTENTIAL IN EVAPORATED SAMPLES

Cathode, silver (3 mm); anode, molybdenum (4 mm); Ag^+ added, 0·25 ml of 10^{-2} M solution; and supporting electrolyte, 0·1 M sulphosalicylic acid

Sample	Amount, ml	E_p, V	i_p, μA	Remarks
Water	10	−0·55	1·50	To *x* ml of the sample, as indicated
Acetone	10	−0·41	1·53	in column 2, contained in a
Acetone	25	−0·41	1·52	beaker, 0·25 ml of 10^{-2} M Ag^+
Dioxan	8	−0·31	1·52	was added, the solution was
Methanol	10	−0·33	1·50	evaporated on a water-bath and
Methanol	50	−0·33	1·50	the residue taken up in 10 ml of
Sodium hydroxide, 1M ..	10	−0·58	1·50	0·1 M sulphosalicylic acid

As indicated in Table I, 0·25 ml of 10^{-2} M silver nitrate was added to varying amounts of the solvents and the solutions were evaporated on a water-bath. The residues were taken up in 10 ml of 0·1 M sulphosalicylic acid. The peak currents obtained for aqueous as well as other solvents were nearly identical, but the peak potentials varied considerably (E_p for dioxan is −0·31 V, for methanol −0·33 V and for acetone −0·41 V; for water and sodium hydroxide solutions it has the same value). When determining silver, it is desirable to confirm the silver peaks by standard addition.

Different volumes (8 to 10 ml) of acetone, dioxan, methanol and sodium hydroxide solution were taken, and the procedure given above was followed. If it is required to determine lower concentrations of silver, then a greater amount of sample (25 to 50 ml) can be taken for evaporation and the residue dissolved in 5 or 3 ml of 0·1 M sulphosalicylic acid instead of 10 ml.

TABLE II

RECOVERY OF SILVER FROM THE VARIOUS SAMPLES BY USING A SEMI-MICRO CELL ON A DIFFERENTIAL CATHODE-RAY POLAROGRAPH

Cathode, silver (3 mm); anode, molybdenum (4 mm); and supporting electrolyte, 0·1 M sulphosalicylic acid

Sample material	Silver added, μg	Silver recovered, μg	Error, per cent.
Methanol	34·0	34·0	Nil
Methanol	13·5	14·4	+6·67
Acetone	34·0	37·0	+8·82
Dioxan	33·0	36·0	+9·09
Sodium hydroxide, 1M	31·5	31·5	Nil

Addition is calculated on 1-g basis for the sample.

As shown in Table II, up to 10 p.p.m. of silver can be determined with an error of ±10 per cent. for the initial sample solutions of 8 to 10 ml. The amount of silver recovered is the mean of three readings. With 50 ml of sample solution taken for evaporation and a final volume of 3 to 5 ml, it was possible to determine silver down to the 1 p.p.m. level. It was confirmed that mercury, even if present in amount three times that of the silver, does not interfere. The silver content of the alkali and solvents was found to be less than 1 p.p.m.

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NOTE—References 4 and 13 are to Parts I and III, of this series, respectively.

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Limit of Determination in Photometric Titrations with Self-indicating Systems*

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An attempt is made to predict the lower limit of concentration (the limit of determination) of a photometric titration based on a self-indicating reaction. When only instrumental factors influence the precision of the result of such a titration it appears to be possible to express the limit of determination in terms of the precision of the absorbance reading and other characteristics of the apparatus (path length and cell volume) and of the reaction ($\Sigma\epsilon$).

The theoretical prediction has been checked by using two commercially available photometers and two reactions, *viz.*, the titration of vanadium(IV) with cerium(IV), and the titration of cerium(IV) with iron(II). The agreement between the theoretically predicted value of the limit of determination and the experimental value appeared to be satisfactory.

The limit of determination is defined in this paper as the amount of substance, or its concentration, that will give a standard deviation of 5 per cent. in the result. A photometric titration with a self-indicating system is based on the light absorption of one or more of the substances involved in the reaction and does not, therefore, require an indicator. In this study we have only investigated the influence of the photometer on the limit of determination. Two commercially available instruments were used, *viz.*, a Zeiss spectrophotometer PMQ II (deflection type) and a Zeiss Elko II filter photometer (substitution principle, especially suitable for the accurate measurement of small absorbances). Simple mathematical expressions are derived for the calculation of the standard deviation in the result of a manual photometric titration in terms of the standard deviation of the absorbance readings of the instrument.

Other factors influencing the precision of the result of a photometric titration, such as chemical kinetics, are not considered.

Finally the predicted limit of determination is compared with experimental results for titrations based on "ideal reactions," in which instrumental errors are greater than chemical errors.

Mathematical expressions—

Let the reaction be

$$X + S \rightarrow XS,$$

where X is the substance to be determined, S the reagent and XS the reaction product.

Assuming that Beer's law is valid for all substances, the absorbance at any moment during the reaction can be expressed by

$$A = b\Sigma\epsilon_1 c_1,$$

where b is the length of the cell and ϵ_1 and c_1 are the molar absorptivity and the concentration of the absorbing substances, respectively. If the reaction proceeds quantitatively to the right and is extremely fast, which occurs in practice, the titration curve will consist of two straight lines, p and q, intersecting at the equivalence point, provided that correction for dilution is carried out.

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The straight line p, before the equivalence point, is given by

$$A = \frac{bt(\epsilon_{xs}-\epsilon_x)}{V} v + C_p \quad .. \quad .. \quad .. \quad .. \quad (1a);$$

the line q, after the equivalence point, by

$$A = \frac{bt\epsilon_s}{V} v + C_q \quad .. \quad .. \quad .. \quad .. \quad (1b),$$

where t is the normality of the standard solution of S; V the volume of the solution at the beginning of the titration; v the volume of the standard solution of S added to the solution; and C_p and C_q are constants of values $\frac{\epsilon_x bv_e t}{V}$ and $(\epsilon_{xs} - \epsilon_s)\frac{bv_e t}{V}$, respectively, where v_e is the volume added at the equivalence point.

If the slope factors of p and q are m_p and m_q, then

$$\Delta m = m_p - m_q = bt\frac{\Sigma\epsilon}{V} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where $\Sigma\epsilon$ represents the sum of the ϵ values, each with the appropriate sign.

In practice p and q lines are found by means of absorbance readings, A, taken after additions, v, of standard solution. Let the co-ordinates of line p be $(x_1,y_1)_p$ and those of line q be $(x_1,y_1)_q$, where x represents volume and y the corrected absorbance. The point of intersection of p and q, corresponding with the end-point volume, v_e, is given by the equation

$$v_e = \frac{\bar{y}_p - \bar{y}_q + m_p\bar{x}_p - m_q\bar{x}_q}{\Delta m} \quad .. \quad .. \quad .. \quad .. \quad (3),$$

where $\bar{y}_p$ is the average of the y-values of line p, etc.

The m-values are obtained by the classical least squares method, with the usual assumptions.[1]

By analogy, the variance in the y-direction can be calculated for each of the lines p and q. The result[2] is

$$s_y^2 = \frac{\Sigma y_i^2 - \bar{y}\Sigma y_1 - m(\Sigma x_1 y_1 - \bar{x}\Sigma y_1)}{N-2} \quad .. \quad .. \quad .. \quad (4),$$

where N is the number of points for p or q.

The variance of y can also be expressed in terms of s_A^2 (the variance in the absorbance reading) and s_v^2 (the variance in additions of the standard solution)—

$$s_y^2 = s_A^2 + m^2 s_v^2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5).$$

When precision burettes are used $s_A^2 \gg m^2 s_v^2$, and $s_y = s_A$.

Applying the law of propagation of errors to v_e in equation (3) and substituting s_A for s_y, the variance in v_e is given by—

$$s_{v_e}^2 = \frac{s_{Ap}^2}{(\Delta m^2)}\left\{\frac{N_p v_e^2 - 2v_e \sum\limits_p x_i + \sum\limits_p x_i^2}{N_p \sum\limits_p x_i^2 - \left(\sum\limits_p x_1\right)^2}\right\} + \frac{s_{Aq}^2}{(\Delta m)^2}\left\{\frac{N_q v_e^2 - 2v_e \sum\limits_q x_i + \sum\limits_q x_i^2}{N_q \sum\limits_q x_i^2 - \left(\sum\limits_q x_i\right)^2}\right\} \quad .. \quad (6).$$

This equation can be simplified. The expressions in brackets in equation (6) are dependent on the number of absorbance readings of the lines p and q, on v_e and on the values of x_1, which can be expressed in terms of v_e as follows. Assuming a regular distribution of the volume additions, x_1, over the whole titration curve, *e.g.*, for five absorbance readings on each of the lines p and q, volumes of 0·1, 0·3, 0·5, 0·7 and 0·9 v_e on line p, and 1·1, 1·3, 1·5, 1·7 and 1·9 v_e on line q, the mean values of the expressions in brackets, for different values of N, are

N_p and N_q	2	3	4	5	6	9
Values of the terms in brackets ..	5·0	2·3	1·5	1·1	0·9	0·5 .

These values are slightly dependent on the position of the various points on the lines p and q.

Five or six points on each line are the usual number of points in practice, and a regular distribution of the points is always taken. Therefore, for five or six regularly distributed points on each of the lines p and q the predicted variance in the end-point is

$$s^2_{v_e} = \frac{1}{(\Delta m)^2}(s^2_{Aq} + s^2_{Aq}) \quad .. \quad .. \quad .. \quad .. \quad (6a).$$

This assumes a constant value of s_A along the lines p and q. This is especially true at the limit of determination, where absorbance changes are small, and s_{Ap} is also equal to s_{Aq}, resulting in

$$s_{v_e} = \frac{s_A}{\Delta m}\sqrt{2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6b).$$

According to our definition, at the limit of determination

$$s_{v_e} = 0{\cdot}05\, v_e \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7).$$

From equations (7), (6b) and (2) the limit of determination, expressed in terms of the amount of substance X, is given by—

$$v_e t = \frac{s_A\sqrt{2}}{0{\cdot}05\, b\Sigma\epsilon} V \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8a),$$

and in terms of the concentration of X by

$$c_X = \frac{s_A\sqrt{2}}{0{\cdot}05\, b\Sigma\epsilon} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8b).$$

The limit of determination depends, therefore, on the instrumental arrangement (s_A, V and b), as well as on the optical properties of the substances involved ($\Sigma\epsilon$).

When only one of the substances involved in the reaction absorbs, the value of the difference in absorbance at the limit of determination can be calculated from equation (8b)

$$\Delta A = \epsilon b c_X = \frac{s_A\sqrt{2}}{0{\cdot}05} \quad .. \quad .. \quad .. \quad .. \quad (9).$$

In practice these predicted limits of determination can be obtained when instrumental errors alone are involved. Any chemical or kinetic influence will disturb the system.

Experimental results are compared with these predicted values with the two photometers for two reactions.

RESULTS OBTAINED WITH THE PMQ II SPECTROPHOTOMETER, DEFLECTION TYPE—

Reproducible values of the standard deviation s_A can only be measured in vibration-free and draught-free rooms. Some scale drift also occurs, especially during the first 2 hours after the lamp has been switched on.

Many absorbance values at $A = 0$ were measured, visually and by photographing the scale at time intervals of 15 seconds, at different values of the amplification of the apparatus and at different wavelengths. It can be proved that no important change in s_A occurs in the absorbance region of $A = 0$ to 0·5.[3] Details are given in Table I.

TABLE I

VALUES OF s^2_A AT $A = 0$ WITH THE PMQ II SPECTROPHOTOMETER AT DIFFERENT WAVELENGTHS AND AMPLIFICATION

Wavelength, nm	Detector	Amplification	S^2_A absorbance units
850	Photocell	0/1/I	$0{\cdot}21 \times 10^{-6}$
600	Multiplier	0/1/I	$0{\cdot}37 \times 10^{-6}$
500	Multiplier	0/1/I	$0{\cdot}73 \times 10^{-6}$
500	Multiplier	10/10/I	$0{\cdot}88 \times 10^{-6}$
360	Multiplier	0/1/I	$1{\cdot}12 \times 10^{-6}$
360*	Multiplier	0/1/I	$0{\cdot}03 \times 10^{-6}$

A tungsten lamp was used as a light source, except at 360 nm,* when a hydrogen lamp was used.

As seen in Table I, with the tungsten lamp s^2_A increases as the wavelength decreases. This can be explained by Planck's law for black radiation, when temperature fluctuations of the wire in the lamp, caused by voltage fluctuations, are assumed to be the main contribution to the fluctuations in A.

The stability of the hydrogen lamp was, in our experiments, superior to that of the tungsten lamp.

To illustrate the concept of limit of determination we chose the reaction of vanadium(IV) with cerium(IV) in 0·5 M sulphuric acid at 750 nm. This is the wavelength of maximum absorption by vanadium(IV) with $\epsilon = 15$; the other substances involved in the reaction do not absorb at this wavelength.

When $b = 2$ cm and $s_A = 4{\cdot}6 \times 10^{-4}$ (see Table I), in equation (8b), the predicted value of the limit of determination is

$$c_x = 4{\cdot}3 \times 10^{-4}.$$

In Table II results are given for three series of titrations in this concentration range.

TABLE II

RESULTS OF THE TITRATION

Titration of $4{\cdot}25 \times 10^{-4}$ M VO_2^{2+} with 0·045 M cerium(IV)

Experiment No.	Series I v_e, ml	Series II v_e, ml	Series III v_e, ml
1	0·081	0·088	0·101
2	0·083	0·097	0·098
3	0·092	0·095	0·103
4	0·081	0·097	0·094
5	0·093	0·106	0·093
6	0·080	0·097	0·095
7	0·087	—	0·101
8	0·096	—	—
9	0·086	—	—
10	0·087	—	—
V_e	0·0866	0·0968	0·0979
s_{ve}, per cent.	6·4	5·9	4·0
ΔA	0·014	0·014	0·014

As seen, there is good agreement between the standard deviation in the experiments with the value of 5 per cent. taken as the starting point.

The limit of determination is, therefore, in perfect agreement with the statistically predicted value.

RESULTS OBTAINED WITH THE ELKO II, FILTER PHOTOMETER, SUBSTITUTION PRINCIPLE—

The same reaction was carried out with the Elko II photometer.

The standard deviation of the absorbance reading at $A = 0$ was 4×10^{-5} for the filter S75,[4] selected for the titration of VO_2^{2+} with cerium(IV).

When $b = 2$ cm, $s_A = 4 \times 10^{-5}$ and $\epsilon = 15$, substituting in equation (8b), the limit of determination is given by

$$c_x = 3{\cdot}8 \times 10^{-5}.$$

Two series of titrations were carried out with solutions of about this concentration. In the first, 8 ml of $11{\cdot}2 \times 10^{-5}$ M VO_2^{2+} were titrated with 4×10^{-3} M cerium(IV). The results for seven titrations were $v_e = 0{\cdot}2299$ ml, $s_{v_e} = 1{\cdot}6$ per cent. and $\Delta A = 0{\cdot}003$.

In the second series the concentration of VO_2^{2+} was $5{\cdot}6 \times 10^{-5}$ M, and for eleven titrations $v_e = 0{\cdot}1281$ ml, $s_{v_e} = 3{\cdot}7$ per cent. and $\Delta A = 0{\cdot}0015$. Again these results agree with the predicted values.

The titration of cerium(IV) with iron(II) was also investigated; using the absorption of cerium(IV) at 490 nm, with filter S49E and $b = 2$ cm, $s_A = 4 \times 10^{-5}$ and $\Sigma_\epsilon = 12$ we predict that

$$c_x = 4{\cdot}7 \times 10^{-5}.$$

Titrations with 4×10^{-5} M cerium(IV) were carried out, and for twelve titrations $s_{v_e} = 5{\cdot}8$ per cent., the absorbance range during the whole titration being about 0·001 absorbance units. This reaction also behaves ideally.

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The Atomic-emission Spectroscopy of the Rare Earth Elements in a Separated Nitrous Oxide - Acetylene Flame*

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Flame emission in the interconal zone of a separated nitrous oxide - acetylene flame is shown to provide a sensitive technique for the detection of the rare earth elements, scandium and yttrium. The relative intensities of the principal atomic lines of these elements emitted in this flame have been measured, and the detection limits at the lines most suitable for flame-emission spectroscopy are reported for aqueous and ethanolic sample solutions. The effect on the atomic-emission intensities obtained for each of the sixteen elements investigated in the presence of the other fifteen elements has been investigated. At the concentrations used in this general survey no serious spectral line interferences between the rare earths were observed because of the simple spectra excited by the hot, reducing fuel-rich flame.

ATOMIC flame-emission spectroscopy has several potential advantages over other flame spectroscopic techniques for the determination of traces of the rare earth elements. This technique has not been adopted for the rare earths as fully as its potential sensitivity and selectivity warrant. Molecular-band emission spectroscopy in flames has long been applied to the rare earth elements, however, and many of the rare earth monoxides emit well defined band spectra in flames of moderate temperature such as the air - acetylene,[1,2] oxygen - hydrogen[3,4] and town gas - oxygen[5] flames. When individual rare earths are to be determined in complex rare earth matrices, however, serious radiational and physical inter-element effects are frequently encountered in analytical molecular-band spectroscopy in such flames. These effects are caused by the complexity of the band spectra observed from rare earth mixtures and the refractory nature of many of the rare earth oxides. The hot, reducing fuel-rich oxygen - acetylene flame has been shown to promote dissociation of rare earth oxide species to provide excited rare earth atoms for analytical atomic spectroscopy.[6,7] Later application of a total-consumption nebuliser - burner fitted with a pre-mixing attachment has further enabled these workers[8,9] to obtain superior detection limits for the rare earths in this flame because of the lower background and noise levels from the oxygen - acetylene flame at this burner. In general, the samples were introduced into the flame as ethanolic solutions of the rare earth perchlorate salts. Skogerboe, Heybey and Morrison[10] have reported atomic emission from alcoholic solutions of several rare earths introduced into a turbulent oxygen - hydrogen flame by a force-feed mechanical delivery system. Pickett and Koirtyohann[11] have investigated the emission of several of the rare earth elements in the pre-mixed nitrous oxide - acetylene flame by using a long-path atomic-absorption burner. In several recent papers from this laboratory separated flames have been demonstrated to possess several advantages over conventional flames in atomic-emission, atomic-absorption and atomic-fluorescence spectroscopy.[12,13,14,15,16] In particular, the interconal zone of the fuel-rich separated nitrous oxide - acetylene flame exhibits a high temperature and has strongly reducing properties. Its reducing nature and relatively low background and noise levels make the interconal region of this stable pre-mixed flame particularly suitable for the atomic-emission spectroscopy of elements that form refractory oxides in cooler conventional flames. This paper describes the application of this flame to the sensitive and selective detection of the rare earth elements.

APPARATUS—

The burner arrangement used has been described in detail elsewhere[15]; it consists of a circular, stainless-steel, water-cooled burner head with a circular slot of 0·50 mm wide and 11 mm i.d. The silica separator tube is 50 mm long and 21·5 mm i.d. A short silica side-arm

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is fused to the separator tube and this is fitted with a B19 ground-glass cone and socket, to which an optical-quality silica end-window is cemented. In operation, the primary reaction zone of the flame burns steadily at the slot of the burner head. The diffusion zone, where the incompletely combusted products of the primary reaction burn with atmospheric oxygen, is maintained at the top of the silica separator tube. The interconal zone of the flame is viewed by the monochromator and detector system via the window in the side-arm of the separator tube. The detector assembly and nebuliser unit of a Techtron AA4 flame spectrophotometer (Varian - Techtron Pty. Limited, Victoria, Australia) were used. The monochromator of this apparatus has a reciprocal linear dispersion at the exit slit, in the first order, of 3·3 nm. The photomultiplier detector was a Hamamatsu, type R213, spectral response 185 to 800 nm, and wavelength of maximum response 430 nm. Nitrous oxide pressure at the nebuliser was 7·5 p.s.i.g., corresponding to a flow-rate of 5·2 litres minute^{-1}. The 1 nm minute^{-1} spectrum scanning motor used in the earlier work[15] was replaced by a synchronous motor to drive the monochromator at 10 nm minute^{-1}.

Reagents—

The rare earths were obtained as their oxides (Johnson, Matthey and Co., London). All solutions were prepared by dilution of 1000 p.p.m. ethanolic stock solutions of the rare earth perchlorates. With the exception of cerium these stock solutions were prepared as follows.

The required amount of the rare earth oxide was transferred to a 10-ml beaker and 8 ml of 60 per cent. analytical-reagent grade perchloric acid added. The solution was evaporated at a distance of about 5 cm below an infrared lamp (250 W, Philips) until first a clear solution formed and then crystals of the rare earth perchlorate were deposited. At this point the solution volume was 0·2 to 0·3 ml. The beaker was then placed in a desiccator containing silica gel to cool. When the solutions were cool crystallisation was complete and no free liquid remained. The crystalline perchlorate was dissolved and diluted with absolute ethanol to 100 ml to yield a 1000 p.p.m. solution, and stored in a polythene bottle.

The cerium stock solution was prepared from AnalaR ammonium cerium nitrate, $(NH_4)_2Ce(NO_3)_6$. The required amount of this salt was weighed into a 25-ml beaker, and perchloric acid was added and the solution evaporated as described above. The evaporation process was repeated twice and the crystals obtained after the third evaporation were dissolved in absolute ethanol in the manner previously described.

A 10,000 p.p.m. sodium solution was prepared by dissolution of sodium perchlorate in absolute ethanol. All rare earth solutions nebulised into the flame contained 200 p.p.m. of sodium.

Results and discussion

To assist interpretation of the flame-emission spectroscopy results, the effect of nebulisation of ethanolic rather than aqueous solutions on the nebuliser efficiency and solution nebulisation rate was first investigated. Samarium solutions in distilled water, 25, 50 and 75 per cent. ethanol - water solutions and absolute ethanol were nebulised into the separated nitrous oxide - acetylene flame at a flow-rate of 5·2 litres minute^{-1} of nitrous oxide. In each instance 10 ml of solution were nebulised several times in the flame and the results obtained are shown in Table I.

Table I

Effect of ethanol on solution nebulisation rate and emission intensity for samarium at 488·3 nm

Solution	Volume entering flame, ml minute^{-1}	Nebulisation rate relative to water	Emission intensity for samarium at 488·3 nm relative to water
Aqueous	0·27	1:1	1:1
25 per cent. ethanol ..	0·31	1:1·2	1:1·4
50 per cent. ethanol ..	0·43	1:1·5	1:1·8
75 per cent. ethanol ..	0·54	1:2·0	1:3·1
100 per cent. ethanol ..	0·62	1:2·2	1:6·5

It is evident that the nebulisation efficiency is doubled when absolute ethanol is used. The final column in Table I shows the manner in which the use of organic solvent affects the emission intensity for samarium at 488·3 nm. Here slightly more than 6-fold enhancement in observed emission intensity is obtained for samarium introduced into the flame in absolute ethanol compared with that observed when an aqueous solution of the same concentration is nebulised. Similar enhancements can be observed for many of the other rare earth elements. This effect cannot be attributed to more efficient nebulisation for ethanol, and it is difficult unequivocally to ascribe the additional enhancement to one particular cause. It may possibly be attributed to a slightly higher flame temperature or increased reducing ability in the interconal zone obtained when an organic solvent is nebulised, or by a chemiluminescent effect whereby a fraction of the rare earth atoms present in the flame plasma is formed directly in the excited state in the presence of organic solvent.

EFFECT OF ADDITION OF SODIUM—

Several authors[17,18] have described the effect of ionisation of atoms of various elements in the nitrous oxide - acetylene flame on their determination by atomic-absorption spectroscopy. Manning,[19] for example, has reported that 75 per cent. ionisation of europium atoms occurs when 100 p.p.m. europium solutions are introduced into a nitrous oxide - acetylene flame. It is common practice in flame spectroscopy to suppress thermal ionisation of this type by the addition of a high concentration of an element such as sodium or potassium, the ionisation potential of which is lower than that of the atoms of the element being determined. The ionisation potentials of the rare earths lie between about 5·5 and 6·5 eV, so that appreciable ionisation occurs for many of the elements in the nitrous oxide - acetylene flame. Lower sensitivities, therefore, result from the smaller number of ground-state neutral atoms available for excitation. The effect of the addition of a more easily ionised element on the atomic emission of a typical rare earth element has been investigated. A 25 p.p.m. ethanolic solution of samarium (ionisation potential 5·6 eV) was used for this investigation. The results obtained are shown in Table II. It was decided from these results that throughout the investigation all rare earth solutions nebulised should contain 200 p.p.m. of sodium to prevent any loss of sensitivity through partial ionisation.

TABLE II

EFFECT OF ADDITION OF SODIUM TO SUPPRESS IONISATION OF SAMARIUM IN THE SEPARATED NITROUS OXIDE - ACETYLENE FLAME

Solution	Emission intensity at 488·3 nm, arbitrary units
25 p.p.m. of samarium	37·5
25 p.p.m. of samarium + 10 p.p.m. of sodium	55
25 p.p.m. of samarium + 50 p.p.m. of sodium	62
25 p.p.m. of samarium + 100 p.p.m. of sodium	66·5
25 p.p.m. of samarium + 200 p.p.m. of sodium	67·5
25 p.p.m. of samarium + 500 p.p.m. of sodium	67
25 p.p.m. of samarium + 1000 p.p.m. of sodium	67

SELECTION OF SUITABLE ATOMIC LINES FOR ANALYTICAL SPECTROSCOPY—

With the exception of cerium and gadolinium, solutions were prepared that contained 100 p.p.m. of the rare earth element under study and 200 p.p.m. of sodium in ethanol. The cerium and gadolinium solutions contained 500 p.p.m. of cerium and 200 p.p.m. of gadolinium. These solutions were nebulised into the separated nitrous oxide - acetylene flame and the atomic-emission spectrum for each element was recorded. The fuel flow-rate was adjusted in each instance to obtain the maximum signal-to-background ratio for each element. For example, the optimum ratio for europium was obtained even from a fuel-lean flame, whereas for cerium and gadolinium a higher background, reducing fuel-rich flame was required to obtain useful atomic emission for these elements. The instrumental conditions used throughout were as follows. Slit width 100 nm (spectral band width 0·33 nm); scan rate 10 nm minute^{-1}; recorder speed 1 inch minute^{-1}; and nitrous oxide flow-rate 5·2 litres minute^{-1}. The amplifier gain was adjusted in each instance to ensure that the emission recorded from the most intense line for the element concerned produced full-scale deflection at the recorder. The most intense useful lines were selected from the recorded spectra, and the lines most suitable for the quantitative determination of the elements were chosen taking into account

the flame background in the same spectral region. The relative intensities of the analytically useful lines were accurately measured at a slit width of 50 nm (spectral band width 0·16 nm). The results obtained are shown in Table III. These readings were obtained by setting the monochromator at the wavelength of each line and nebulising the sample and blank solutions.

TABLE III

RELATIVE INTENSITIES OF THE MOST USEFUL RARE EARTH EMISSION LINE OBTAINED IN THE SEPARATED NITROUS OXIDE - ACETYLENE FLAME

(In each instance the most intense line of an element is given a relative intensity of one hundred)

Slit 50 μm

Element	Wavelength, nm	Relative intensity*
Lanthanum	550·134	100
	494·977	72
	545·515	60
	428·026	31
Cerium	520·012†‡	100
	520·042‡	
	520·039‡	
Praseodymium	495·136	100
	513·342	56
	493·974	52
	492·459	46
Neodymium	492·453	100
	463·424	41
	468·345	39
	494·483	35
	495·478	50
Samarium	488·377	130
	476·027	75
	444·181	75
	444·228	
	520·059	68
	478·310	62
	471·610	60
	471·707	
	428·221	35
	428·283	
	428·350	
Europium	459·403	100
	462·722	77
	466·188	61
	576·520	4
Gadolinium	434·662	100
	451·966	80
	432·712	72
	422·585	72
	442·241	59
	440·186	54
	405·822	50
	443·063	47
	407·870	43
	441·473	36
	430·634	33
	431·384	17
Terbium	432·647	100
	431·885	67
	433·845	62
	406·159	25
	449·308	25
Dysprosium	421·172	100
	418·678	46
	404·599	46
	419·485	31
	458·937	11
Holmium	410·384	100
	405·393	82
	416·303	50
	404·081	5
Erbium	400·797	100
	415·110	58
	397·304	27
	397·360	
	408·765	16
	460·662	9
Thulium	410·584	100
	418·762	94
	409·419	93
	371·792	78
	374·407	47
	435·993	26
	530·712	19
	567·585	12
Ytterbium	398·798	100
	346·436	9
	555·648	8
Lutetium	451·857	100
	351·211	34
	337·650	26
	328·174	22
	327·897	14
Scandium	391·181	100
	402·369	91
	390·749	83
	402·040	71
	399·661	11
	326·991	5
Yttrium	407·738	100
	410·238	92
	412·831	78
	412·485	71
	464·370	43
	467·484	34
	362·094	25
	404·764	11

* Uncorrected for response characteristics of Hamamatsu R213 photomultiplier and monochromator.

† These are the only clearly defined assignable lines observed for cerium.

‡ Denotes unresolved lines.

LIMITS OF DETECTION—

Table IV shows the detection limit obtained in the separated nitrous oxide - acetylene flame with the instrumentation described here in 10, 25, 50, and 75 per cent. ethanol - water mixtures and in absolute ethanol for each of the rare earth elements. The detection limit was defined as that concentration of the element in solution producing a signal-to-noise ratio of unity. A slit width of 50 nm was used to obtain these results. The use of this relatively narrow band-pass (0·16 nm) yields poorer detection limits in many instances than when a wider slit was used, but ensures minimal spectral interference from atomic lines of other elements present in the matrix to be analysed. The optimum fuel flow-rate was used for each element when its detection limit was determined. Atomic emission from the elements whose oxides are refractory in nature (*e.g.*, cerium, praseodymium and gadolinium) was at a maximum in a fuel-rich reducing flame. For the less refractory rare earth elements, whose atoms are more readily formed by a purely thermal dissociation of the monoxide species, it was found that lower detection limits were obtained by using a leaner and hotter, less reducing flame.

TABLE IV

RARE EARTH DETECTION LIMITS IN SEPARATED NITROUS OXIDE - ACETYLENE FLAME

		Detection limits, p.p.m.			
Element	Wavelength, nm	Ethanol, 100 per cent.	Ethanol, 75 per cent.	Ethanol, 50 per cent.	Ethanol, 10 per cent.
La	550·134	4	6	8	10
Ce	520·012 520·042 520·039	50	—	—	—
Pr	495·136	1·8	4	10	12·5
Nd	492·453	0·5	0·8	1·2	2·0
Sm	488·377	0·18	0·3	0·4	0·5
Eu	459·403	0·007	0·015	0·017	0·02
Gd	434·662	3	6	8	10
Tb	432·647	1·3	2·8	6	7·5
Dy	421·172	0·18	0·25	0·46	0·55
Ho	410·384	0·07	0·18	0·23	0·30
Er	400·797	0·21	0·32	0·45	0·5
Tm	410·584	0·06	0·13	0·17	0·25
Yb	398·798	0·012	0·019	0·025	0·033
Lu	451·857	0·7	1·0	1·4	1·7
Sc	391·181	0·06	0·10	0·13	0·16
Y	407·738	0·5	0·65	0·77	0·9

INTERFERENCES—

The effect on the atomic-emission intensity obtained at the selected analytical lines for each of the sixteen elements investigated in the presence of the other fifteen elements was examined. A slit width of 500 nm (band width 0·16 nm) was used throughout to minimise spectral interference. Initially it was hoped that improved selectivity might result from adjusting the acetylene flow-rate to suit the element under study. For example, spectral interference from any nearby atomic lines of the more refractory rare earth elements on the chosen analytical line of a less refractory element can be minimised by using a stoicheiometric flame. Under these conditions, however, more serious interference may be encountered from the oxide band spectra of refractory rare earth elements than in a fuel-rich flame, and this replaces the interference caused by their atomic emission. This effect was observed in several instances. The acetylene flow-rate corresponding to that required for a slightly fuel-rich flame was, therefore, used throughout. The following procedure was used. Solutions containing twenty times the minimum detectable concentration of the element studied, or 100 p.p.m., whichever was less, were prepared. These solutions were prepared to contain 200 p.p.m. of sodium and 100 p.p.m. of the rare earth element, the interference of which was under examination. Each of these solutions was nebulised in turn in the separated flame, followed by a standard solution of the element at the same concentration (containing no interfering element) and a blank solution. The degree of any interference was assessed from the signals obtained for the standard solution and solution of the ion containing the foreign ion. In each instance the monochromator was set at the wavelength of the most useful analytical line. When serious interference was encountered, an attempt was made to find an alternative line for the element at which the foreign ion did not interfere. All

the investigations of interferences were carried out with a 50 per cent. ethanol - water mixture. The results obtained are shown in Table V.

TABLE V

SPECTRAL INTERFERENCES FOUND FOR DETERMINATION OF EACH ELEMENT INVESTIGATED

Element, p.p.m.	Wavelength, nm	Interfering species and wavelength of line, nm	Magnitude of interference for 100 p.p.m. of interfering ion, per cent.
Lanthanum, 100	550·134	—	—
Cerium, 300	520·042	—	—
Praseodymium, 100	495·136	Nd 495·029 Nd 495·067 Nd 495·246	4
	513·342	Nd (100 p.p.m.) does not interfere	—
Neodymium, 25	492·453	Pr 492·459	35
		Sm 492·404	80
	463·424	Pr and Sm do not interfere	
Samarium, 8	488·397	Nd 488·381	200
		Y oxide band	10
		Sc oxide band	25
	471·610	Nd and Sc do not interfere	
		Y	3
Europium, 0·34	459·403	Gadolinium oxide band interferes	20
		The 462·722 and 466·188 europium lines are also in a gadolinium oxide band system and cannot be used as an alternative	
Gadolinium, 100	434·646	—	—
Terbium, 100	432·647	Gd 432·712	10
	431·885	Gd does not interfere	
Dysprosium, 7·5	421·172	—	—
Holmium, 4·5	410·384	Y 410·238	35
		Dy 410·388	12
	405·392	Y and Dy do not interfere	—
Erbium, 9	400·797	—	—
Thulium, 3·4	410·584	Ho 410·384	10
	499·418	Ho does not interfere	
Ytterbium, 0·5	398·798	Er 398·766	40
	346·436	Er does not interfere	
Lutetium, 28	451·857	Pr 451·663	10
		Gd 451·966	10
	331·211	Pr and Gd do not interfere	
Scandium, 2·5	391·181	—	—
Yttrium, 14	407·738	Gd 407·870	3
		Er 407·788	5
	410·238	Er and Gd do not interfere	

It was observed that several of the rare earth oxide samples used during this work contained detectable amounts of other rare earth elements. In these instances an apparent positive interference effect results when the element occurring as impurity in the sample whose interference is being investigated is the same as that being determined. This was corrected for by nebulising a solution of the interfering ion and subtracting the observed line-emission intensity at the wavelength of the analytical line of the element being determined.

At the concentrations investigated it is evident that in only one case (samarium) do as many as three other rare earth elements interfere when the most useful analytical line is used. For praseodymium, neodymium, samarium, terbium, holmium, thulium, ytterbium, lutetium and yttrium even these elements which interfere at the most useful line do not interfere when an alternative (but usually less intense) line is selected. Lanthanum, gadolinium, dysprosium, erbium and scandium can be determined at the concentrations investigated without interference in the presence of any other rare earth elements at 100 p.p.m.

CONCLUSION

The principal difficulty encountered in the development of both flame-emission and absorption methods for the determination of the rare earth elements has until recently been the lack of suitable flames capable of dissociating the extremely stable rare earth monoxide species formed in most flames. Fassel, Curry and Kniseley[6] have demonstrated the usefulness

of the hot, fuel-rich oxygen - acetylene flame in this respect for production of the atomic spectra of the rare earths. These authors concluded that in this flame thermal dissociation of the monoxide molecules does not appear to be the primary process in populating the flame with neutral (or excited) atoms. The dissociation of rare earth monoxide molecules in the pre-mixed nitrous oxide - acetylene flame reported here also requires a fuel-rich flame. As is also the case for the oxygen - acetylene flame, the fuel-rich flame is slightly cooler than the stoicheiometric flame, and if the dissociation mechanism were purely thermal in nature, greater line intensities would be expected in the stoicheiometric flame. The necessity for the use of a fuel-rich nitrous oxide - acetylene flame has also been demonstrated in the determination of the rare earth elements by atomic-absorption spectroscopy.[20] Several workers[21,22,23] have suggested mechanisms to explain the need for fuel-rich reducing flames to dissociate molecular oxide molecules.

The investigation reported here reveals that good sensitivity is available for the determination of the rare earth elements by flame-emission spectroscopy in the separated nitrous oxide - acetylene flame. The use of a separated flame ensures suppression of flame background radiation from OH and the chemiluminescence of the reaction $CO + O \rightarrow CO_2$. The reducing atmosphere in the interconal zone of the fuel-rich flame is also protected from atmospheric oxidation in this flame, and this results in the presence of a greater total number of emitting sample atoms whose radiation is available for detection. Because of the high intensity of the emitted radiation from samples introduced into the flame, it is possible to retain good sensitivity while using a monochromator of good resolution at narrow slit widths to obtain good spectral selectivity. The sensitivities obtained compare well with those obtainable by flame-emission spectroscopy in the oxygen - acetylene flame at a total-consumption atomiser - burner, and even in only 10 per cent. ethanol - water solutions the detection limits obtained for neodymium, europium, holmium, lutetium, scandium, samarium, ytterbium and lanthanum are equal or superior to those obtainable by atomic-absorption spectroscopy.[20] Good selectivity is easier to obtain by measurement of the atomic-line spectra of the rare earths by flame-emission spectroscopy than by arc or spark-emission spectroscopy. The simpler spectra obtainable with the flame source as compared with the often complex spectra from arc and spark sources enable analyses of rare earth mixtures to be made with a simple low dispersion spectrophotometer. Even with a high resolution spectrograph the positive location of lines of the element required to be determined is frequently difficult, and the incidence of interference from lines of other rare earth elements is high.

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Emission Spectra Obtained from the Combustion of Organic Compounds in Hydrogen Flames

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A study is presented of the emission spectra produced by nebulisation of organic liquids into a nitrogen - hydrogen diffusion flame burning in air, and into a laminar-flow pre-mixed air - hydrogen flame. Both flames exhibit low background emissions and that of the diffusion flame is particularly low even over the OH-band region. Carbon, hydrogen and oxygen-containing compounds exhibit intense bands for CH, CHO, C_2 and OH species. Nitrogen-containing organic compounds additionally display NH, NO and CN bands; chlorine-containing compounds give CCl bands in the diffusion flame; sulphur compounds give CS and S_2 bands; and phosphorus compounds give HPO and PO bands.

The spectral distribution of these bands and their intensities indicate that many types of organic compound can be characterised and identified by direct observation of their emission spectra during combustion in these cool flames.

The measurement of the flame-emission spectra of organic compounds is a relatively new technique in analytical chemistry, but Gaydon[1] has already identified many emitting species (C_2, CH, CHO and CH_2O) from flames supported by various organic compounds.

Flame-emission detectors for use in gas chromatography have been described.[2,3,4] These detectors involve the use of the C_2 and CH emission from air or oxy-hydrogen flames. McCormack[5] found that greater sensitivities were obtained by using a microwave-generated plasma rather than the flame as a method of identifying peaks from gas chromatograms.

More recently McCrea and Light[6] have described the measurement of C_2 and CH-band emissions for the determination of hydrocarbons in methanol, and Robinson and Smith[7] have given a more general description of the emission spectra of organic liquids in oxy-hydrogen flames.

The purpose of this study was to investigate the emission spectra produced by combusting organic compounds containing nitrogen, chlorine, phosphorus and sulphur, in addition to carbon, hydrogen and oxygen, in low-temperature laminar-flow nitrogen - hydrogen diffusion flames and pre-mixed air - hydrogen flames.

Apparatus—

For these studies a Unicam SP900A flame-emission - atomic-absorption spectrophotometer, fitted with an E.M.I. 9601B photomultiplier, a standard air - acetylene (rectangular) burner head and various quartz tube burner heads (see text), was used. The spectra were recorded with a Servoscribe recorder used over the 0 to 10-mV range.

Fuel gas, hydrogen from a cylinder; diluent gas, nitrogen from a cylinder; and air from a compressor.

Results

Emission from the nitrogen - hydrogen diffusion flame—

This flame, which has previously been described[8,9,10,11] in papers from this laboratory, was maintained on the standard Unicam 1·8 × 7·5-cm air - acetylene emission head. The only background emission from this flame corresponds to OH-band emission about 310 nm, and even this is only about one fortieth of that of a conventional pre-mixed air - hydrogen flame.

Nitrogen was used as the nebulising gas (at 15 p.s.i.g., 4 l minute^{-1}) with the conventional SP900A nebulising system. Hydrogen was introduced, as usual, at the bottom of the burner. The CH and C_2 emissions, when nebulising methanol, increased as the hydrogen flow-rate increased, but at fairly high flow-rates the emission began to decrease. The nebulisation of certain organic compounds (*e.g.*, benzene) caused the flame to become unstable at even moderate hydrogen flow-rates. The most stable, reproducible flame was obtained when the hydrogen flow-rate was set just above that necessary to prevent the flame from lifting off when nebulising distilled water.

Emission measurements were taken with the top of the burner head level with the bottom of the monochromator slit. Thus measurements were taken from the coolest region of the flame[8] (centre temperature about 280° C, outer temperature about 800° C) where least breakdown would be expected to occur and fairly large molecular fragments should exist. The thermal energy of the flame is insufficient to account for all of the observed emissions, most of which are of chemiluminescent origin. Solid organic compounds were not examined at this stage because it was necessary to dissolve them in various organic solvents and concomitant complication of the observed spectra. Solutions were, however, used with the air - hydrogen flame.

CARBON - HYDROGEN AND CARBON - HYDROGEN - OXYGEN COMPOUNDS—

Table I summarises the band emissions seen during the aspiration of methanol, acetone, acetylacetone, isopropyl alcohol, benzene and formaldehyde into the diffusion flame. Obviously CH and CHO compounds predominantly yield C_2 and CH emission spectra and increase the OH-band emission.

TABLE I

BANDS OBSERVED FROM SOME REPRESENTATIVE C–H–O COMPOUNDS AND BENZENE

Compound	Bands observed*	Main wavelengths, nm	Most intense band
Methanol	C_2	516	CH
	CH	431	
Acetone	C_2	516	CH
	CH	431	
Isopropyl alcohol	C_2	516	CH
	CH	431	
Acetylacetone	C_2	516	CH
	CH	431	
Benzene	C_2	516	C_2
	CH	431	
Formaldehyde (40 per cent. aqueous)	Complex banded continuum	390 to 570	—
	CH	431	—

* OH emission was observed at about 310 nm for all compounds and the intensity was about 2 to 4 times that caused by the flame background.

Methanol—It will be seen from Fig. 1 that in the region 400 to 500 nm the only emissions observed from the combustion of methanol were CH (431 nm) and C_2 (main band head at 516 nm). The fact that the latter is almost as strong as the former, although the methanol molecule contains only one carbon atom, suggests complex exothermic mechanisms involving liberation of excited C_2 species. The OH emission about 310 nm is four times greater than for water. The continuum, which has an intensity of about one half that of the C_2 band, may be caused by the CO - O_2 reaction or other unidentified breakdown products.

Acetone—The emission from acetone (also isopropyl alcohol and acetylacetone) was similar to that for methanol except that, relative to the C_2 band and the continuum, the CH band was more pronounced. The OH emission was about twice that observed for methanol.

Benzene—The C_2 emission was, as expected, more intense and was greater than the CH emission. Two weak bands at 274 and 267 nm caused by undecomposed benzene were discernible above the OH background, and emission of the latter at 310 nm was similar in intensity to that for methanol.

Formaldehyde solution (40 *per cent., aqueous*)—The C_2 emission was negligible and the CH emission at 431 nm was surprisingly weak. A broad complex band was observed from 390 to 570 nm.

It can be concluded that the CH : C_2 ratio is not significant because the emissions are chemiluminescent and critically dependent on fuel flow-rate and nebulisation rate.

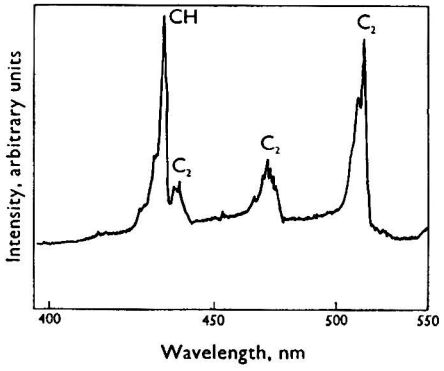

Fig. 1. The spectrum of methanol in the nitrogen - hydrogen diffusion flame: slit, 0·02 mm; gain, 3,10; band width, 2

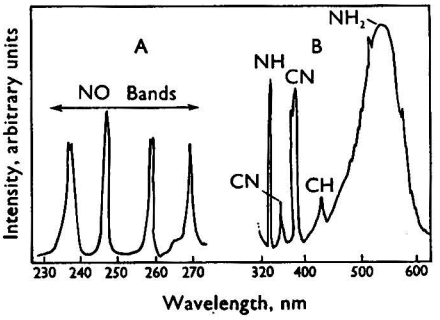

Fig. 2. The spectrum of 50 per cent. methanol - 50 per cent. 0·88 ammonia solution v/v in the nitrogen - hydrogen diffusion flame: A, slit, 0·03 mm; 3,5; band width, 2. B, slit, 0·10 mm; gain, 3,8; band width, 2

NITROGEN-CONTAINING COMPOUNDS—

All organic nitrogen-containing compounds tested gave CN, NH and NO emissions. The CH : CN : NH : NO intensity ratio (see Table II) would appear to be dependent to some degree on the structure of the compound. It is, however, important to note that, as with CH and O compounds, these ratios are dependent on the hydrogen and nitrogen flow-rates and the rate of nebulisation, as well as the optical system used. It was essential to maintain the gas flow-rates constant during these measurements.

TABLE II

CN : CH : NH : NO EMISSION INTENSITY RATIOS OF NITROGEN-CONTAINING COMPOUNDS IN THE NITROGEN - HYDROGEN DIFFUSION FLAME

Compound	Emission intensity* CN,† 360 nm	CH, 431 nm	NH, 337 nm	NO, 259 nm
0·88 Ammonia solution - methanol (1 + 1 v/v)	100	93	250	300
Acetonitrile	100	77	40	94
Butylamine	100	390	78	160
Diaminoethane	100	92	77	170
Pyridine	100	210	50	140

* The CN, CH and NH emission intensities were all measured with a slit width of 0·03 mm and converted into gain 2,5. The NO emission was measured at slit width 0·1 mm and converted into gain 3,5. The CN : CH : NH : NO ratio was then calculated after arbitrarily setting the CN emission intensity to 100 units.

† The main 389 nm CN band was not used because it was four times more intense than the 360 nm CN band and made the measurement of the CN : CH : NH ratios difficult.

Ammonia (50 per cent. 0·88 ammonia solution - 50 per cent. methanol v/v)—The main features of this spectrum (see Fig. 2) were a broad band extending from 600 to 330 nm with a maximum at 520 nm, weak CN and NH emissions peaking at 389 and 337 nm, respectively, and weak NO emission[12] with main band heads at 215, 227, 237, 248, 259 and 272 nm. Weak C_2 and CH emissions were observed at 516 and 431 nm, respectively. The broad band from 600 to 330 nm was attributed to NH_2 emission.[12]

The CN emission must result from a chemiluminescent reaction between the methanol and ammonia (or breakdown products of methanol and ammonia). The NO-band emission which extended into the far ultraviolet must be caused by an energetic reaction because bands about 220 nm require an excitation energy of 5·5 eV, which is far beyond the energy available in the diffusion flame. As the NO bands were still present on nebulising aqueous ammonia solution, we must assume that some energetic reaction between nitrogen and oxygen species must occur at the edge of the flame to produce excited NO molecules, and that carbon species are not necessary.

Acetonitrile—Weak C_2 and CH emissions were observed at 516 and 431 nm. The CH emission at 431 nm was 2·5 times more intense than the C_2 emission at 516 nm. The most prominent features of the spectrum were the intense CN bands at 360, 389 and 418 nm (see Fig. 3). Weak NO bands at 215, 227, 237, 248, 259 and 272 nm were also observed.

Pyridine, butylamine and diaminoethane—Spectra similar to that of acetonitrile were obtained. The only major difference was that C_2 and CH emissions were much stronger with respect to the CN emission and that the NH:CN intensity ratio had increased (see Table II).

CHLORINE COMPOUNDS—

Carbon tetrachloride—The main features of this spectrum were intense C_2 emission and weak CH and CCl emission at 277 and 279 nm (Fig. 4). The intense C_2 emission is difficult to explain as the carbon tetrachloride molecule contains only one carbon atom, but can be accounted for by re-combination processes.

Chloroform—The chloroform spectrum was similar to that of carbon tetrachloride, except that the C–H emission was slightly more intense with respect to the C_2 emission.

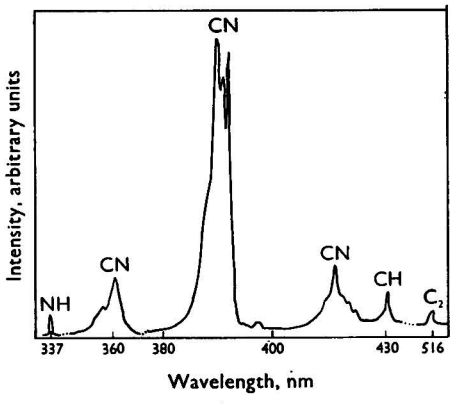

Fig. 3. The spectrum of acetonitrile in the nitrogen - hydrogen diffusion flame: slit, 0·013 mm; gain, 2,0; band width, 2

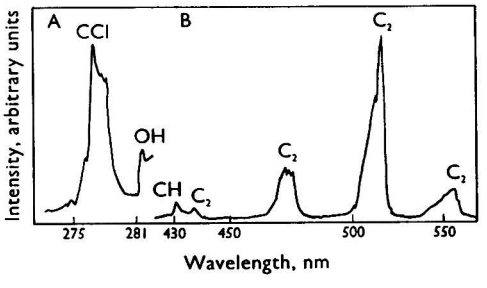

Fig. 4. The spectrum of carbon tetrachloride in the nitrogen - hydrogen diffusion flame: A, slit, 0·01 mm; gain, 3,0; band width, 2. B, slit, 0·038 mm; gain, 3,10; band width, 2

EFFECT OF ADDING AIR TO THE NITROGEN - HYDROGEN FLAME—

The effect of adding air through a third jet in the burner base[13] was to increase the C_2, CH, NH, CN, NO and OH emissions, but to decrease the emission from C–Cl.

THE LAMINAR-FLOW PRE-MIXED AIR - HYDROGEN FLAME—

Previous workers[6,7] have used total-consumption burners which gave turbulent air and oxy-hydrogen flames. The organic emission extended throughout the flame. In the present study, when methanol was nebulised into a laminar-flow pre-mixed air - hydrogen flame, well defined blue primary reaction cones were observed just above the holes in the burner head. It is from these primary reaction cones that almost all the observed organic emission occurs. In general, the maximal emission intensity for most species occurred in the lean flame

with the hydrogen flow-rate just above the flash-back point. Under these conditions with the standard burner head used, the primary reaction cones were about 3 mm high. The length of these cones could be increased to 18 mm to fill the monochromator slit by increasing the hydrogen flow-rate, but this resulted in a decrease in the emission intensity.

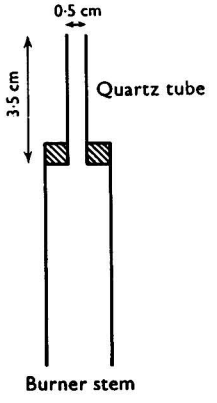

Fig. 5. The laminar-flow pre-mixed air - hydrogen burner head

Instead of using the standard burner head, the flame could be maintained more suitably on a 5 mm i.d. quartz tube fitted into the top of the burner stem with a suitable adaptor (Fig. 5). When methanol was nebulised, a single, well defined, primary reaction cone, 18 mm high, was obtained with the hydrogen flow-rate just above the flash-back point.

This "tube" burner showed no carbon build-up, unlike conventional burner heads, and also remained quite cool in operation. Larger diameter "tube" burners (*i.e.*, 10 mm) gave weaker emission and a tendency to flash-back, while smaller diameter burners (*i.e.*, 3 mm) caused the flame to lift off rather easily. In general, the emission intensities with the 5-mm tube burner were about 2 to 3 times greater than those obtained with the conventional burner head.

The organic compounds under test were nebulised as 10 per cent. v/v solutions in methanol. This procedure was adopted for two reasons. Firstly, the flame was rather unstable when nebulising certain compounds (*e.g.*, benzene and pyridine) and, secondly, the nebulising properties of solutions containing 90 per cent. v/v of methanol should be similar. In fact the rate of solution uptake was found to be similar for a range of compounds (see Table III). The spectra from the organic compounds are always accompanied by those from methanol in these experiments.

TABLE III

RATE OF UPTAKE OF METHANOLIC SOLUTIONS

Compound	Rate of solution uptake,* ml minute^{-1}
Methanol	3·05
Acetonitrile, 10 per cent.	3·19
Butylamine, 10 per cent.	2·94
Triethylamine, 10 per cent.	2·94
Pyridine, 10 per cent.	2·97
Nitrobenzene, 10 per cent.	2·88

* About 0·7 ml minute^{-1} actually reaches the flame.

The emission from the air - hydrogen flame was quite dependent on the hydrogen flow-rate and this was always set just above the flash-back point when nebulising methanol. (Under these flame conditions the CH and CN emission intensities were optimised, while optimal C_2 emission occurred at a slightly higher hydrogen flow-rate.) The air pressure was set at 15 p.s.i.g. (4 l minute^{-1}) by using the conventional Unicam nebulising system.

The C_2 and CH emissions from methanol constituted about 5 to 60 per cent. of the total C_2 and CH emission observed from the 10 per cent. sample - 90 per cent. methanol solutions. Thus, not surprisingly, the C_2:CH ratio of a given substance was not meaningful.

The background emission of the methanol solution was low at the main CN, NH and NO peaks. The OH emission at 310 nm was fairly constant (±20 per cent.) for all solutions examined. Methanol gave a spectrum similar to that from the diffusion flame (Fig. 1); the only major difference was that there was less of a continuum beneath the C_2 and CH peaks. The CH emission at 431 nm was only about eight times more intense than in the diffusion flame. This low ratio was surprising because of the considerable temperature difference between the two flames, and underlines the chemiluminescent nature of the emission. Acetone in methanol gave a spectrum similar to methanol, but the C_2 emission was more pronounced, while with benzene in methanol only C_2 and CH emissions were observed. Indeed, all compounds examined which contained only carbon, hydrogen and oxygen gave similar spectra, exhibiting C_2, CH and OH emissions.

NITROGEN COMPOUNDS—

The ammonia in methanol gave a spectrum similar to that observed in the diffusion flame (Fig. 2), but far more intense, with NH_2, NH and CN emission and weak C_2 and CH emission. The spectrum from acetonitrile in methanol was also similar to that in the diffusion flame (Fig. 3). The CN emission intensity at 389 nm when nebulising undiluted acetonitrile was fifteen times more intense than that from the diffusion flame, while the CH emission at 431 nm was only about six times more intense. With butylamine in methanol, the major difference was the increase in the NH:CH intensity ratio.

All nitrogen compounds gave CN, NH and NO emission, the only significant differences between the spectra were the actual CN:NH:NO emission intensity ratios, and these are shown in Table IV.

TABLE IV

CN:NH:NO EMISSION INTENSITY RATIOS OF NITROGEN-CONTAINING COMPOUNDS IN THE PRE-MIXED AIR - HYDROGEN FLAME

Compound	Emission intensity* CN, 360 nm	NH, 337 nm	NO, 259 nm
0·88 Ammonia solution - methanol (1 + 1 v/v)	100	280	800
Acetonitrile	100	38	40
Butylamine	100	89	126
Diaminoethane	100	64	90
Pyridine	100	24	31
Diethylamine	100	62	67
Triethylamine	100	51	67
Nitrobenzene	100	107	270

* The CN and NH emission intensities were all measured with a slit width of 0·02 mm and converted into gain 2,10. The NO emission was measured at slit width 0·08 mm and converted into gain 3,10. The CN:NH:NO ratio was then calculated after arbitrarily setting the CN emission intensity to 100 units.

It would appear that the CN:NH ratio increases in the order ammonia < primary < secondary < tertiary amines, while the CN:NO ratio increases in the order ammonia < RNO_2 < RNH_2 < R_2NH ≈ R_3N < RCN <  N; *i.e.*, the CN:NO intensity ratio is high for compounds containing multiple carbon - nitrogen bonds and low for compounds containing  and NH_2 groupings.

The CN emission at 389 nm from acetonitrile in methanol was directly proportional to its concentration over the range 0·005 to 10 per cent.

CHLORINE COMPOUNDS—

The flame assumed an intense green colour on aspiration of $CHCl_3$ and CCl_4 and intense C_2 and weak CH emissions were observed. No CCl emission was observed as in the diffusion flame, *loc. cit.*

SULPHUR COMPOUNDS—

With carbon disulphide in methanol, the flame assumed an intense purple colour and a broad continuum from about 570 to 250 nm, and a peak about 385 nm, were observed (Fig. 6). Above the continuum, some weak S_2 emission was present. Weak CS emission at 257 nm and extremely weak C_2 and CH emissions were also observed. The thiophen spectrum showed only C_2 and CH emission, the C_2 emission was intense compared with the CH emission. No S_2 or CS emission was observed. This could be caused by incomplete breakdown of the thiophen in the primary reaction cone of the air - hydrogen flame.

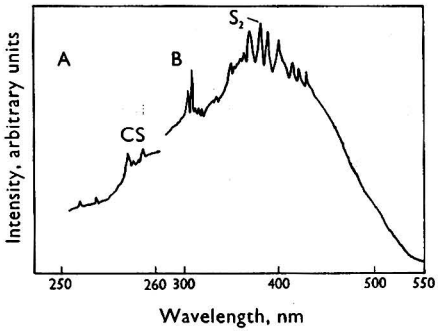

Fig. 6. The spectrum of 10 per cent. carbon disulphide - 90 per cent. methanol v/v in the air - hydrogen flame: A, slit, 0·012 mm; gain, 3,4; band width, 2. B, slit, 0·02 mm; gain, 3,10; band width, 2

PHOSPHORUS COMPOUNDS—

A strong continuum with a peak about 530 nm was observed between 600 to 400 nm (Fig. 7) on aspirating tributyl phosphate solution. This was attributed to the HPO species.[12,14,15] Weak C_2 and OH emissions were observed above this continuum. In addition, strong PO emissions were observed in the ultraviolet, with main bands at 240, 246, 247, 248, 254 and 256 nm. The emission intensity at the most intense PO band at 246 nm was

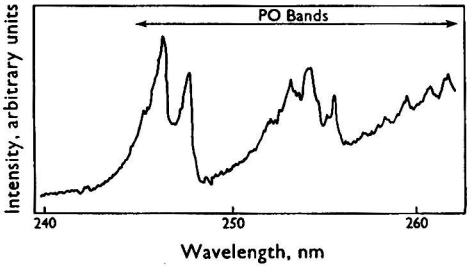

Fig. 7. The spectrum of 10 per cent. tributyl phosphate - 90 per cent. methanol v/v in the air - hydrogen flame: slit, 0·04 mm; gain, 3,10; band width, 2

proportional to the tributyl phosphate concentration in methanol over the range 0·05 to 10 per cent. No CP emission[12] was observed. When phosphoric acid (0·01 per cent. in methanol) was aspirated, strong HPO emission was observed in the visible and PO in the ultraviolet.

CONCLUSIONS

It is important to stress that the emissions from both flames are chemiluminescent in origin and are, therefore, markedly dependent on experimental variables in their intensities.

The intensities from the diffusion flame were, in general, an order of magnitude lower than those obtained with the pre-mixed air - hydrogen flame. It is possible, however, that too much sample was reaching the flame and quenching the emission, because on nebulising methanol the inner temperature of the flame decreased.[8]

Although the pre-mixed air - hydrogen flame generally gives higher sensitivities, it possesses a higher background and there are certain instances when spectra, *e.g.*, S_2, can be obtained in the diffusion flame, which can scarcely be observed in the pre-mixed air - hydrogen flame.

The emission intensities from this flame should be greatly increased if undiluted compound were nebulised instead of 10 per cent. methanolic solutions. It should be possible to monitor the output of a gas-chromatographic column by using this type of flame without, however, encountering the nebulisation problem experienced in this study.

These experiments show that many organic compounds can be typified by spraying them into one of these two flames and observing the simple band spectra thus obtained. When compounds of similar composition have been separated by gas chromatography, identification and quantitative measurement should be possible. Quantitative relationships were in fact observed for acetonitrile in methanol and tributyl phosphate in methanol wih the air - hydrogen flame.

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A Method for the Determination of Lead in Blood by Atomic-absorption Spectrophotometry

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A method for the determination of trace amounts of lead in blood is described. The organic material in the blood is oxidised by dry ashing at 500° C and a solution of the ash in dilute hydrochloric acid prepared. The lead in the ash solution is determined by atomic absorption at 217 nm in an air - propane flame after isolation by a double-extraction procedure with dithizone and ammonium tetramethylenedithiocarbamate as complexing agents. Recovery tests are carried out and the lead content of the blood of workers from a lead mine, determined by this method, is compared with results obtained by using the mixed colour method of the Analytical Methods Committee. Comparative tests with wet-oxidation and dry-ashing techniques are made on samples of blood to which known amounts of lead have been added. The interference caused by bismuth is also investigated.

THE determination of lead in blood is an important factor in the assessment of the toxicological hazard to workers engaged in lead mining.[1] Workers exposed to lead dusts or fumes usually absorb some lead and generally have raised blood-lead levels.[2] Kehoe[3] has stated that an individual with a blood-lead content in excess of 80 μg per 100 g of blood is in danger of developing lead intoxication. Accurate methods of analysis are, therefore, required for the indication of abnormal lead absorption. The lead content of the blood of workers in lead mines in Western Ireland is constantly monitored[1] and more than three thousand determinations have been carried out in this laboratory over the past 2 years. As a result of these tests, workers with excessively high lead contents in their blood are placed under clinical observation and hospital treatment provided if symptoms of lead poisoning develop.

Published methods for the determination of lead in blood generally involve destruction of the organic material followed by determination of lead as dithizonate.[4,5,6] Polarographic,[7] spectrographic[8,9] and atomic-absorption spectrophotometric[10,11] methods have also been used for determination of lead in blood and other biological materials. In this connection the reports and recommendations of Hoffman[12,13] with regard to the need for further study on methods for the determination of lead in food by atomic-absorption spectrophotometry are noted.

For the determination of lead in blood, digestion with concentrated acids is usually preferred to dry ashing[4,5,6] but, as dry ashing is more economical in time and reagents, several tests were carried out to compare the results obtained with both methods. The investigation showed that although wet oxidation is more accurate, the dry-ashing method is quite acceptable for routine work involving many samples. In this respect it is noted that in a collaborative study[14] on the determination of trace metals in animal feeds by atomic-absorption spectrophotometry comparable results were obtained with both wet-digestion and dry-ashing methods for the destruction of the organic material.

Several methods for the determination of lead in the blood ash solutions by atomic absorption were tried, including direct aspiration of a solution of the ash in dilute hydrochloric acid, single extraction of lead dithizonate into isobutyl methyl ketone, and also single extraction of the complex formed between lead and ammonium tetramethylenedithiocarbamate into isobutyl methyl ketone. However, more consistent recovery results were obtained with both dithizone and ammonium tetramethylenedithiocarbamate in a double-extraction procedure. In this method, the lead is extracted into dithizone in chloroform from an ammoniacal solution containing citrate and cyanide, returned to nitric acid and finally extracted into isobutyl methyl ketone as its complex with ammonium tetramethylenedithiocarbamate. This method gives satisfactory agreement with the mixed colour method of the Analytical Methods Committee.[15]

Disposable 5-ml plastic syringes are used to take the samples of blood from the mine workers, and the method described in this paper is designed for the determination of lead in about 5 ml of blood. The detection limit with this method is 2 μg per 100 g of blood (0·02 p.p.m.).

Experimental

Apparatus—

Atomic-absorption spectrophotometer—A Perkin-Elmer, model 303, atomic-absorption spectrophotometer equipped with a hollow-cathode lead lamp and a three-slot Boling burner is used. The operating parameters are: compressed air flow-rate 6 ml minute^{-1}; wavelength 217 nm; slit position 4; lamp current 30 mA; solution uptake 4·2 ml minute^{-1}; scale expansion × 10; and meter response 2. The propane flow-rate is adjusted during aspiration of water-saturated isobutyl methyl ketone to give a blue flame in which "three tongues of flame" are visible.

Muffle furnace—The furnace should be capable of operating at 500° C.

Silica basins—These should be lead-free with a capacity of about 50 ml, and have a lip.

Separating funnels—Capacity, 50 ml.

Graduated flasks—Capacity, 50 ml.

Reagents—

All reagents must be lead-free, and water must be distilled or de-ionised and lead-free.

Hydrochloric acid, sp.gr. 1·18.

Dilute hydrochloric acid—Dilute 10 volumes of hydrochloric acid (sp.gr. 1·18) to 100 volumes with water.

Nitric acid, sp.gr. 1·42.

Dilute nitric acid—Dilute 1 volume of nitric acid (sp.gr. 1·42) to 100 volumes with water.

Ammonia solution—Dilute 1 volume of ammonia solution (sp.gr. 0·880) with 1 volume of water.

Chloroform—Analytical-reagent grade.

Potassium cyanide solution—A 10 per cent. w/v solution in water.

Dithizone (diphenylthiocarbazone)—Analytical-reagent grade.

Concentrated dithizone solution—A 0·3 per cent. w/v solution in chloroform. Filter and store in a refrigerator.

Dilute dithizone solution—Dilute 1 volume of concentrated dithizone solution to 100 volumes with chloroform. Prepare freshly as required.

Concentrated lead standard solution—Dissolve 1·60 g of lead nitrate in water, add 10 ml of nitric acid (sp.gr. 1·42) and dilute to 1 litre with water.

Dilute lead standard solution—Dilute 1 volume of concentrated lead standard solution to 1000 volumes with dilute nitric acid solution.

1 ml of solution ≡ 1 μg of lead.

Thymol blue indicator solution—Triturate 0·4 g of thymol blue with 8·6 ml of 0·1 N sodium hydroxide and dilute to 1 litre with 95 per cent. ethanol.

Acetic acid - sodium acetate solution—Dissolve 15 g of anhydrous sodium acetate in water, add 21 ml of glacial acetic acid and dilute to 1 litre with water.

Ammonium tetramethylenedithiocarbamate solution—A 1 per cent. w/v solution in water.

Isobutyl methyl ketone—Water-saturated isobutyl methyl ketone.

The following additional apparatus and reagents are required for the wet-oxidation method.

Digestion flasks—Kjeldahl transparent Vitreosil flasks.

Sulphuric acid, sp.gr. 1·84.

Perchloric acid, sp.gr. 1·54.

Sample preparation

Reagent blank—

Carry out a blank test on all the reagents omitting only the sample.

Destruction of organic matter—

Weigh the sample of blood into a silica basin, evaporate to dryness on a boiling water bath, heat over a low flame to volatilise as much of the organic material as possible and then

in a muffle furnace at 500° C for 4 hours. (This is normally sufficient for complete ashing, but if any charred organic material remains it can be removed by the addition of a slight excess of dilute hydrochloric acid solution, then evaporating to dryness on a boiling water bath and again ashing at 500° C for 30 minutes.) Remove the dish from the furnace, allow it to cool, place on a boiling water bath, add 2 ml of hydrochloric acid (sp.gr. 1·18), heat for 3 minutes, add 20 ml of water, heat for a further 5 minutes, transfer it to a 50-ml graduated flask, wash the silica dish with distilled water, add the washings to the flask and dilute to 50 ml with distilled water.

SEPARATION OF LEAD—

Transfer 25 ml of the sample obtained by the method described above under Sample preparation to a separating funnel, add 3 drops of thymol blue indicator solution, 3 ml of ammonium citrate solution and sufficient ammonia solution to give a green - blue colour, indicating pH 9·0 to 9·5. Add 1 ml of potassium cyanide solution and 5 ml of dilute dithizone solution; shake the funnel for 1 minute and allow to separate. Run the chloroform layer into a second separating funnel and reject the aqueous layer. Add 10 ml of dilute nitric acid solution to the chloroform extract, shake for 1 minute, allow it to separate and reject the chloroform layer. To the nitric acid layer, add 9 ml of acetic acid - sodium acetate solution. Adjust the pH to the range 2·2 to 2·8 by the addition of dilute nitric acid solution or acetic acid - sodium acetate solution, by using pH paper (see Note 1). Add 1 ml of ammonium tetramethylenedithiocarbamate solution and 5 ml of isobutyl methyl ketone solution; shake the mixture for 2 minutes, allow it to separate and reject the aqueous layer. Filter the organic layer through a 7-cm Whatman No. 541 filter-paper into a test-tube. Set the zero on the atomic-absorption spectrophotometer with the blank solution and measure the percentage absorption of the sample solution. Convert the percentage absorption into absorbance and read the number of micrograms of lead from a calibration graph.

PREPARATION OF CALIBRATION GRAPH—

Measure 0, 0·5, 1·0, 1·5, 2·0 and 3·0 ml of dilute lead standard solution into separating funnels, dilute to 10 ml with dilute nitric acid solution and proceed as described under Separation of lead, beginning at the words "add 9 ml of acetic acid - sodium acetate solution. . . ." Plot a graph relating the number of micrograms of lead to absorbance. The absorption graph is linear.

A separate calibration graph is prepared for each batch of samples and standard solutions are introduced at regular intervals during the aspiration of the test samples to ensure that the instrument is operating with a constant sensitivity.

NOTE 1—

pH adjustment is not usually necessary because the addition of 9 ml of acetic acid to 10 ml of dilute nitric acid solution gives a pH of about 2.6. The pH of this mixed solution should be checked after preparation.

RESULTS

Several samples of blood of low lead content, each weighing 5 g, were ashed in the manner described. The ash was dissolved in hydrochloric acid and known amounts of lead added to each ash solution. The lead content of each solution was determined separately on four occasions. The results obtained are shown in Table I.

TABLE I

RECOVERY OF LEAD ADDED TO BLOOD ASH SOLUTIONS

Lead added, μg per 100 g of blood	Lead found after subtraction of blank, μg per 100 g of blood			
10	11	8	10	9
20	20	17	19	21
30	29	28	30	29
40	37	40	41	38
60	58	60	59	59
80	81	80	79	81
100	102	99	100	98

The lead content of the blank sample of blood was 7 μg per 100 g.

INTERFERENCE CAUSED BY BISMUTH—

As bismuth may cause some interference in the determination of lead by the mixed colour method[15,16] a series of tests was carried out to investigate the effect of bismuth on the determination of lead by the atomic-absorption method. Known amounts of lead and bismuth were, therefore, added to blood ash solutions and the lead content determined by the method described. Each sample was analysed in duplicate and the results obtained are shown in Table II.

TABLE II

RECOVERY OF LEAD ADDED TO BLOOD ASH SOLUTIONS CONTAINING BISMUTH

Lead added, μg per 100 g of blood	Bismuth added, μg per 100 g of blood	Lead found after subtraction of blank, μg per 100 g of blood (1)	(2)
10	0	8	10
10	10	9	10
10	25	11	9
10	50	10	12
10	100	9	8
20	0	21	18
20	10	19	20
20	25	20	17
20	50	18	21
20	100	19	21
40	0	39	41
40	10	40	41
40	25	37	39
40	50	38	41
40	100	39	42
80	0	78	81
80	10	79	80
80	25	77	78
80	50	78	82
80	100	76	79

The lead content of the blank sample of blood was 9 μg per 100 g.

The results in Table II indicate that the presence of bismuth does not interfere with the determination of lead. No difference in absorption could be detected between the samples containing lead and bismuth and those containing lead alone.

COMPARISON OF ATOMIC-ABSORPTION AND THE ANALYTICAL METHODS COMMITTEE'S MIXED COLOUR METHODS—

The lead content of the blood of 150 workers in lead mines in western Ireland was determined by using both the atomic-absorption spectrophotometric method and the mixed colour method of the Analytical Methods Committee[15] on aliquot samples. Some of the results obtained are shown in Table III.

TABLE III

COMPARISON OF RESULTS OBTAINED BY ATOMIC-ABSORPTION AND MIXED COLOUR METHODS

Worker No.	Atomic-absorption method, μg per 100 g of blood	Mixed colour method, μg per 100 g of blood
1	9	7
2	9	10
3	14	16
4	17	17
5	23	22
6	24	25
7	32	30
8	36	36
9	40	42
10	52	49
11	54	51
12	67	66
13	64	65

The figures in Table III are representative of the results obtained and show that comparable agreement is obtained between both methods.

COMPARISON OF RESULTS OBTAINED BY WET OXIDATION AND DRY ASHING—

Known amounts of lead were added to several duplicate blood samples, aliquot portions of which were dry ashed and wet oxidised. Sulphuric, nitric and perchloric acids were used in the wet oxidation and any precipitate that formed after the oxidation was dissolved by boiling with 10 ml of dilute hydrochloric acid. The lead content of the duplicate samples was determined and the results obtained are shown in Table IV.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY WET-OXIDATION AND DRY-ASHING METHODS

Lead added, μg per 100 g of blood	Lead recovered after subtraction of blank, μg per 100 g of blood	
	Wet-oxidation method	Dry-ashing method
10	9	9
20	19	18
40	38	38
60	58	57
80	78	76
100	96	95

The lead content of the blank sample of blood was 10 μg per 100 g.

The results in Table IV show that the dry-ashing procedure gives recoveries that, on average, are slightly lower than those obtained with the wet-oxidation process. The recovery results compare favourably with those obtained by Gorsuch[17] and the slight loss of lead is probably caused by co-precipitation and solubility factors in the wet-oxidation method, and volatilisation and retention on the silica crucibles in the dry-ashing procedure. The reproducibility factor with such minute amounts of lead must also be taken into consideration.

CONCLUSIONS

A method for the determination of trace amounts of lead in blood has been described. This method is shown to give consistent results and to be suitable for routine control work when the possibility of occupational hazards arise with regard to lead contamination. The presence of bismuth in blood does not cause interference in this method of analysis.

We wish to express our appreciation for the co-operation received from the mining authorities concerned, who were most anxious to facilitate the work with technical and financial assistance.

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Atomic-absorption Determination of Strontium in a Standard Plant Material: Comment on Results of Inter-laboratory Comparison

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It is claimed that the concentration of strontium in Bowen's standard kale (*Analyst*, **1967**, **92**, 124) is 101·13 ± 2·38 p.p.m., on evidence that X-ray fluorescence values obtained subsequently to Bowen's inter-laboratory comparison were in good agreement at 101 p.p.m. with atomic-absorption values obtained in this laboratory by using anion exchange and the addition method, but not reported separately from other atomic-absorption results by Bowen. Methods used in obtaining the neutron-activation results and these other atomic-absorption results implied in the inter-laboratory comparison should be examined for serious interferences.

Because of the value of 101 p.p.m. for strontium obtained by Champion and Whittem[1] in their X-ray fluorescence analysis of Bowen's standard kale, it has become necessary to reveal the original atomic-absorption values contributed by this laboratory to Bowen for inclusion in his inter-laboratory comparison of results.[2] These are shown in Table I.

Table I

Atomic-absorption values for strontium in standard kale derived from separate portions of the powdered material and corrected to oven-dry basis (80° to 90° C for 18 hours)

By addition method, p.p.m.—				Mean
103·4	104·6	100·4	101·4	102·4
103·6	97·2	97·7	101·0	99·9
Against separate standards, p.p.m.—				
105·5	105·6	105·2	107·0	105·8

Method

For the first eight values in Table I, strontium was determined on 2-g portions of the powdered material received from Dr. Bowen, by ashing in a muffle furnace, dissolving the ash in hydrochloric acid, De-Acidite FF (acetate form) anion-exchange treatment of this solution and atomic-absorption analysis of the column effluent as described by David.[3] The anion-exchange step was effected by using the same column and procedure as that used by David.[4]

The last four values in Table I were obtained from a comparison of atomic-absorption readings carried out on the effluents from the anion-exchange columns with those carried out on separate strontium standards in 0·1 N acetic acid. Absorption was measured at 460·7 nm and an air - acetylene flame was used (10-cm single slot, pre-mix burner).

A moisture value of 5·61 per cent. (mean of twelve determinations) was used to convert the strontium values into the oven-dry basis shown in Table I.

Discussion

Bowen's inter-laboratory study[2] gives a grand mean for strontium of 84·1 ± 10·7 p.p.m. (twenty determinations), a neutron-activation mean of 74·7 ± 4·2 p.p.m. (two laboratories, six determinations) and an atomic-absorption mean of 88·1 ± 10·2 p.p.m. (two laboratories, fourteen determinations). The atomic-absorption mean of 88·1 p.p.m. included all eight of the addition method results shown in Table I, but not the results arising from the use of

separate standards (personal communication from H. J. M. Bowen). A high result from the use of separate standards containing no addition of an easily ionised element is to be expected on grounds of repression of ionisation of strontium by large excesses of potassium and calcium in the sample solutions.

Champion and Whittem's replicates for strontium were 101 and 101 p.p.m. These, together with the eight addition method replicates in Table I, give a value for strontium in the standard kale of 101·13 $\pm$ 2·38 p.p.m. In addition to the results reported here, six other apparently reliable X-ray and flame-emission values in the range 100 to 106 p.p.m. of strontium in the standard kale have been reported to the author in a personal communication from R. N. Whittem.

The atomic-absorption values included in this mean of 101·13 p.p.m. are unlikely to be in error by more than a few parts per million, because severely depressing anions were replaced with acetate and any slight remaining enhancements or depressions compensated for by the use of the addition method. It is suggested, then, that the methods used to produce the other atomic-absorption values and the neutron-activation values for strontium reported in Bowen's inter-laboratory study[2] should be examined for depressive interferences amounting to 20 to 40 per cent. In the case of the atomic-absorption method it is likely that either no interference-suppressing agent, or an inadequate one, was added to the solutions.

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The Determination of Silicon in Intact Steel Samples with a Low Output Neutron Generator

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A low output, sealed-tube neutron generator has been used to determine the silicon content of steels, counting and flux monitoring being carried out with simple equipment.

A consequence of the penetrating nature of neutrons and γ-photons is that neutron-activation analysis involving γ-ray spectroscopy can be used, in certain instances, for elemental analysis of intact samples. This avoids the labour and complication of classical analytical procedures. A radioisotope source or a neutron generator may provide a suitable source of neutrons when there is no access to a nuclear reactor. In particular, neutron generators utilising the reaction

$$^{3}H + ^{2}H \longrightarrow ^{4}He + ^{1}n + 17{\cdot}6\ MeV$$

have found application in analytical laboratories, as high yields of neutrons can be produced at relatively low accelerating voltages (down to 100 kV).[1] Although development of neutron generators has understandably been directed towards improving their neutron output and hence the sensitivity of the analytical methods based on their use, the determination of a number of high cross-section elements at useful levels, particularly in large samples, is possible with low output machines, thus simplifying shielding arrangements and reducing capital outlay necessary for equipment. In addition, operation and maintenance, two further factors influencing the acceptance of an instrumental method in an analytical laboratory, make no greater demands on laboratory staff than the more widely used techniques of instrumental analysis. However, so far low output generators have found few applications in activation analysis, but in this paper we describe the use of such a system, together with very simple counting equipment, for the determination of silicon in intact steel samples.

Experimental

The neutron generator used for this work (K-tube generator, Elliott Electronic Tubes Ltd.) was a pulsed machine operating at a maximum pulse rate of about 3 pulses s^{-1} and with a total neutron output of about 10^8 neutrons s^{-1}. As neutrons were emitted from a sealed-tube unit, no vacuum or pumping system was necessary and neutron output was adjusted by setting two controls, tube pressure and H.T. Radioactivity in the sample was detected with a 3 × 3-inch sodium iodide scintillator, and analogue pulses from the detector were fed, after amplification, to a pulse height analysing system; initially pulse height distribution was examined with a 128-channel analyser, but subsequently quantitative results were obtained with a single-channel analyser to reduce the over-all cost of the counting assembly.

Samples were prepared from turnings of steels of known silicon content by pressing into pellets, 0·75 inch diameter × 0·1 inch, with a small hydraulic press; standards were made up from mixtures of silicon in iron in the same way.

Activity induced in 0·005-inch thick copper foils was used to monitor the flux falling on the sample during irradiation, to obviate the need for further electronic equipment. Cups, the correct size to enclose the pressed samples, were prepared from the foils with a shaping tool, but copper discs placed on top of the pellets were also found to give reliable results, although in the latter case greater care was required to align foil and sample correctly during irradiation.

Results and discussion

The reaction of silicon with generator neutrons to form aluminium-28 by the reaction ^{28}Si (n,p) ^{28}Al is well known. The reaction has a cross-section of 220 mb and, as a result of decay of the product aluminium-28 (half-life 2·3 minutes), a γ-line with a characteristic energy of $E\gamma = 1{\cdot}78$ MeV is emitted, which is suitable for measurement. Nuclear interferences in the analytical determinations are provided by the production of aluminium-28 from phosphorus and aluminium by the reactions ^{31}P (n,α) ^{28}Al and ^{27}Al (n,γ) ^{28}Al; the ratios of specific activities induced in silicon, phosphorus and aluminium under the conditions used in the work were 1 : 0·55 : 0·0062, respectively. The levels of both phosphorus and aluminium in the mild-steel samples analysed were sufficiently low not to introduce serious error into the measurement. The effect of alloying elements is small and has been considered in detail elsewhere.[2] The lower limit of the concentration of silicon that could be reliably determined was imposed by the intensity of the 1·81-MeV γ-line from manganese-56 formed by the (n,p) reaction on the iron matrix, as the scintillation counter could not distinguish between this γ-line and the 1·78-MeV γ-line used for quantitative determination of the silicon content of the steel. Under the conditions of irradiation, cooling and counting used in this work, the yield of the two lines was similar at levels of silicon in iron of about 0·15 per cent.

Samples and standards were irradiated individually, and the activity induced in the copper foil used to calculate a correction factor to compensate for flux variations that occurred from run to run. After the completion of an irradiation, first the sample (or standard) then the copper foil were counted with the single-channel analyser set to accept pulses corresponding to γ-lines of 1·8 and 0·51 MeV, respectively. The 1·8-MeV counts from the sample were measured again after sufficient time had elapsed to permit the short-lived aluminium-28 activity to die away, so that correction could be made for the contribution of manganese-56 activity to the initial count from the sample. Total irradiation and counting time was not more than 10 minutes.

Results obtained are given in Table I.

Table I

Determination of silicon in steels by fast-neutron activation analysis

Sample No.	1	2	3	4	5	6	7	8	9	10	11
Known silicon content, per cent.	0·15	0·25	0·26	0·28	0·35	0·41	0·57	0·63	0·75	0·81	0·96
Silicon content found, per cent.	0·14	0·29	0·27	0·28	0·36	0·41	0·55	0·68	0·76	0·83	0·99

Precision of the determinations, as given by the coefficient of variation on a 0·6 per cent. silicon sample, was found to be better than 5 per cent.

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Nitrate Determination in Soil Extracts with the Nitrate Electrode

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This paper deals with investigations on nitrate determination with a nitrate-specific ion electrode. Analytical results for the nitrate content in soil extracts are compared with analyses carried out by two colorimetric methods. The extracting agents used are 0·02 N copper sulphate, 2 N potassium chloride and distilled water. Results for nitrate determinations in soil suspensions and corresponding filtrates are also compared.

The stability of soil extracts, the influence of variation in the ratio of grams of soil to millilitres of extracting agent and in the shaking time, interferences caused by ionic strength and anions, accuracy and reproducibility are also discussed.

The investigation shows that nitrate determination with the electrode is reliable both for soil extracts and for soil suspensions. The determination is rapid, and the interferences are not serious.

NUMEROUS distillation and colorimetric methods for nitrate determinations in soil extracts have been described.[1 to 14] They are often time consuming or the interferences are serious.

Over the past few years several commercially available ion-selective electrodes have appeared. They are differentiated by the type of membrane which they incorporate.[15] In the nitrate-selective electrode, the membrane is replaced by a liquid ion exchanger, which is insoluble in water. If this electrode is placed in a solution containing nitrate, the potential can be measured against a standard calomel electrode. This potential is a measure of the nitrate activity. By comparison with potentials measured in standard nitrate solutions, it is possible to determine the nitrate content in unknown solutions. The electrode responds to nitrate ion from 0·1 to 10^{-5} M. Potterton and Shults[16] have published an evaluation of the performance of this electrode. Paul and Carlson[17] have studied nitrate determination in plant extracts by the nitrate electrode. After writing this article, we also noticed that Myers and Paul[18] had published some investigations on nitrate determination in soils.

The Orion nitrate-ion activity electrode appears to be convenient for nitrate determinations in soil extracts. It would be of interest to ascertain this by comparing determinations made with this electrode with determinations made with the colorimetric xylenol method[19] and a Technicon AutoAnalyzer method.[20] (These two were chosen because they are used for routine analysis in our laboratory.)

In this investigation, different types of soil and extracting solutions were used. The results from different analytical methods are compared.

EXTRACTING SOLUTIONS—

Various extracting agents have been proposed for nitrate determinations in soils. Bremner[21] recommends 2 N potassium chloride, because all of the nitrate will be extracted and no enzymatic (or chemical) reactions leading to changes in the nitrate content have been observed. The chloride concentration will be high for measurements with the nitrate electrode and for the xylenol method, however, although it will not affect nitrate determinations with the AutoAnalyzer. Water is a suitable extracting agent for the determinations of nitrate by all three methods, but some biological loss of nitrate may occur, although Lewis[11] has shown that this can be prevented by the use of 0·02 N copper sulphate. In view of this, and because the selectivity constant for SO_4^{2-} is low (Orion electrode, 6×10^{-4}), this extracting agent seems to be suitable for the determination of nitrate by the electrode. The xylenol method and the AutoAnalyzer method do not suffer from interference by the copper sulphate in the concentrations used. We decided to use water and copper sulphate as extracting agents and 2 N potassium chloride for the AutoAnalyzer method.

EXPERIMENTAL

APPARATUS—

A specific-ion meter, Model 401, Orion Research, 11 Blackstone Street, Cambridge, Mass., with a nitrate-ion activity electrode was used for the potentiometric nitrate determinations in water and copper sulphate extracts. The measurements were made against a standard calomel electrode. As the slope of the curve millivolts *versus* concentration is not constant below 2 to 3 p.p.m.,[16] the nitrate content cannot be read directly from the meter. A standard graph must, therefore, be drawn for the lower concentrations.

A Technicon AutoAnalyzer, with a manifold system according to Henriksen and Selmer-Olsen,[20] was used for colorimetric determinations of nitrate in water, copper sulphate and potassium chloride extracts.

A Beckman spectrophotometer, Model B, with 1-cm cells was used for colorimetric measurements in the xylenol method.

REAGENTS—

All reagents were of analytical-reagent grade.

The following extracting solutions were used.

Distilled water.

Potassium chloride, 2 N—Dissolve 149 g of potassium chloride in water and make up to 1 litre.

Copper sulphate, 0·02 N—Dissolve 2·5 g of $CuSO_4.5H_2O$ in water then make up to 1 litre.

Standard solutions of nitrate—Stock solutions of nitrate were prepared by dissolving 3·6092 g of potassium nitrate in water and diluting to 1 litre. This is equivalent to 500 μg ml^{-1} of nitrate-nitrogen. Standard nitrate solutions in the range 0 to 15 p.p.m. of nitrogen were made up by diluting appropriate volumes of the stock solution with water, 0·02 N copper sulphate or 2 N potassium chloride.

Reagents for the xylenol method[19]—2,4-Xylenol, ammoniacal silvern itrate solution, bromocresol green indicator, tungstophosphoric acid solution and sulphuric acid.

Reagents for the AutoAnalyzer method[20]—A solution of sulphanilamide in hydrochloric acid, and *N*-1-naphthylethylenediamine dihydrochloride solution.

METHOD

Samples of air-dried soil (5 g) were shaken for 1 hour with 100 ml of the extracting solution. The extracts were filtered through Schleicher and Schüll No. 589 white ribbon filters. The extracts were stored in polythene bottles at room temperature. Water and 0·02 N copper sulphate extracts were used for the nitrate electrode and xylenol method, and water, 0·02 N copper sulphate and 2 N potassium chloride were used for the AutoAnalyzer method.

RESULTS AND DISCUSSION

STORAGE OF EXTRACTS—

Water, 2 N potassium chloride and 0·02 N copper sulphate were used to prepare soil extracts of different types of soil for nitrate determination. The filtered extracts were divided

in two parts, one stored at room temperature (20° C) and the other at 4° C in a refrigerator. The nitrate contents of the copper sulphate and potassium chloride extracts were constant for at least 3 weeks. Some of the water extracts stored at room temperature showed a decrease in the nitrate content.

COMPARISON OF METHODS AND EXTRACTING AGENTS—

A series of soil extracts was prepared, and the nitrate contents of the filtrates were determined by the nitrate electrode, the xylenol method and the AutoAnalyzer method. The results are shown in Table I. A *t*-test did not give any significant difference between the results from the nitrate electrode and the xylenol method with the copper sulphate extract.

TABLE I

DETERMINATION OF NITRATE IN SOIL EXTRACTS

A 5-g sample of air-dried soil was shaken with 100 ml of extracting solution for 1 hour. The figures are expressed as mg of nitrate-nitrogen per litre of extracting solution

Soil sample No.	Soil type	Loss on ignition, per cent.	0·02 N Copper sulphate		2 N Potassium chloride
			electrode	xylenol method	AutoAnalyzer
1	Sand, large organic matter content	15·9	21·0	21·6	21·0
2	Sand, large organic matter content	15·4	17·7	18·8	18·8
3	Sandy peat soil	29·8	13·1	14·2	14·0
4	Peat soil	87·7	5·2	4·7	4·7
5	Peat soil	91·6	14·6	14·7	15·0
6	Peat soil	90·3	30·2	30·1	30·5
7	Peat soil	85·7	2·0	1·3	1·3
8	Peat soil	91·9	23·0	24·5	24·8
9	Peat soil	90·8	38·9	38·0	39·3
10	Peat soil	78·4	1·6	1·5	1·5
11	Peat soil	91·2	48·0	50·0	49·8
12	Peat soil	89·8	63·8	63·3	67·2

TABLE II

CONCENTRATION OF NITRATE-NITROGEN IN VARIOUS SOIL EXTRACTS, mg litre^{-1}

Soil sample No.	Soil type	Loss on ignition, per cent.	0·02 N Copper sulphate	2 N Potassium chloride	Distilled water
1	Peat soil	90·0	0·40	0·40	0·35
2	Peat soil	91·9	0·13	0·15	0·15
3	Peat soil	91·8	1·3	1·4	1·2
4	Peat soil	85·7	0·18	0·30	—
5	Peat soil	91·2	0·10	0·20	0·10
6	Peat soil	91·5	10·7	10·5	9·9
7	Peat soil	79·4	0·20	0·25	0·20
8	Peat soil	92·5	—	0·15	0·10
9	Peat soil	90·2	133	132	129
10	Clayey peat soil	29·1	23·4	23·0	23·2
11	Sand (large) medium organic matter content	17·4	31·9	32·5	31·7
12	Sandy peat soil	30·8	69·2	67·0	65·8
13	Clay moraine	7·9	1·1	1·2	1·2
14	Clay moraine	6·5	0·45	0·55	0·45
15	Sandy moraine	7·0	0·35	0·45	0·33
16	Sandy moraine	6·6	0·70	0·85	0·80

Further, there was no significant difference between the nitrate electrode and the Auto-Analyzer methods when either copper sulphate or potassium chloride was used as extracting solution.

Sixteen different air-dried soil samples, in which both the nitrate and the humus contents showed large variation, were extracted with water, 0·02 N copper sulphate and 2 N potassium chloride. The extracts were filtered and analysed by all three methods the next day, and the agreement between the methods was good. If the nitrate-nitrogen content was below 2 mg l^{-1}, however, the electrode gave higher results than the other two methods, especially for the organic soils. Below 0·5 mg l^{-1} of nitrate-nitrogen there was little deflection on the instrument. The xylenol method should not be used for concentrations below 1 mg l^{-1} of nitrate-nitrogen without including a concentration stage. With the AutoAnalyzer method, the lower limit of detection is about 0·02 p.p.m.

Table II shows the results from the analysis of the sixteen samples. The results are the mean values by all three methods for those with relatively high nitrate content (Nos. 6, 9, 10, 11 and 12). There was good agreement between the nitrate contents of the different extracts. When the nitrate-nitrogen content is below 2 p.p.m., only the results from the AutoAnalyzer method are given. *t*-Tests show that there is a small, but significant, difference between the extracting agents for low values, 2 N potassium chloride is slightly more efficient than copper sulphate for small nitrate-nitrogen contents. For practical purposes, however, both solutions could be used.

Determinations of nitrate in soil suspensions—

It would be of great practical value if it were possible to measure the nitrate content directly in soil suspensions with the nitrate electrode. This would considerably facilitate the determination of the nitrate status of soils. The filtering of peat and clay soils, however, might be tedious.

There seemed to be no significant difference between the nitrate determinations of the soil suspensions and of the filtrates of the same suspensions, either with 0·02 N copper sulphate or water as extracting agents.

Nitrate recovery—

Three soils were selected, and 5 g of air-dried samples were shaken with 100 ml of 0·02 N copper sulphate for 1 hour. The nitrate content in the soil suspensions was determined with the Orion electrode. A small amount of potassium nitrate, corresponding to 2·5 mg of nitrate-nitrogen, was added to each of the suspensions and the nitrate content re-determined. It was found that the increase was nearly 100 per cent. of the added nitrate, namely, 98·8, 97·6 and 98·8 for the three soils.

Variation of the ratio of soils to extracting agents (grams to millilitres)—

In the heavily fertilised soil from greenhouses there appears to be sufficient nitrate present to allow determination at the ratio of 5 g of soil to 100 ml of extracting agent. For organic soils, it is not possible to increase the ratio of soil to solution, because the soil may then absorb all of the liquid. Mineral soils are often poor in nitrate, but in this instance it is possible to increase the amount of soil, and thus increase the nitrate content in the soil suspension.

Two clay soils and two sandy morainic soils were selected for nitrate determinations in soil suspensions with various soil-to-extracting agent ratios. The ratios (g to ml) were 5 : 50, 10 : 50, 20 : 50, 30 : 50 and 50 : 50. The extracting agent was 0·02 N copper sulphate and the shaking period 1 hour. At the ratio 5 : 50, the nitrate content in the suspension was too small to allow determination with great accuracy, but for the ratios 10 : 50 and 20 : 50 the nitrate contents were high enough, and the results agreed well with the values previously obtained with the AutoAnalyzer when the ratio was 5 : 100. By using the higher ratios, 30 : 50 and 50 : 50, the values decreased slightly when expressed as milligrams of nitrate-nitrogen per 100 g of dry soil. This was probably caused by a suspension effect. The nitrate content in the suspension, however, still showed an increase. By using the ratio 20 : 50 it is possible to determine contents as low as 0·4 mg of nitrate-nitrogen per 100 g of air-dried soil, corresponding to 9 lb $acre^{-1}$ (1 kg $dekar^{-1}$), with sufficient accuracy.

VARIATION IN SHAKING TIME—

As the soil particles usually have a negative charge, the anions will not be fixed. Hence nitrate is readily extracted both with salt solutions and distilled water. Kelly and Brown[22] found that shaking it for 5 minutes gave as good results as shaking for 8 hours, and Riehm[23] found that a shaking time of 5 minutes was as efficient as 45 minutes. These results were confirmed by our investigations. Three different soils were extracted for 2, 5, 15, 30 and 60 minutes with copper sulphate, and the nitrate content in the suspensions was determined by the nitrate electrode.

The results in Table III show that almost all of the nitrate is extracted after 2 minutes. The content in the soil suspension of sample 1 is slightly lower than after a longer period of shaking. In rapid determinations of soil nitrate content, however, a shaking time of 2 minutes would be sufficient. Water is also suitable as extracting agent, but the determinations must be carried out immediately to avoid biological loss.

TABLE III

VARIATION IN SHAKING TIME

Soil sample No.	Soil type	Loss on ignition, per cent.	Nitrate-nitrogen in soil extract, p.p.m. Shaking period, minutes 2	5	15	30	60
1	Peat soil	89·8	67·0	68·0	71·0	71·0	70·0
2	Clayey peat soil	29·1	22·8	23·0	24·2	23·2	23·6
3	Sand, large organic matter content	17·4	32·0	31·0	32·0	31·0	31·0

VARIATION IN IONIC STRENGTH—

The measured potential of the nitrate activity depends on the ionic strength of the solution because the activity coefficient decreases with increasing ionic strength. The ionic strength can be determined by measuring the electrical conductance. Table IV shows results obtained for 20 p.p.m. of nitrate-nitrogen in solution and varying copper sulphate concentrations. The instrument was standardised with 0·02 N copper sulphate solutions containing various amounts of nitrate.

TABLE IV

DETERMINATION OF NITRATE AT VARIOUS IONIC STRENGTHS

Nitrate-nitrogen in solution, p.p.m.	Normality of copper sulphate in solution	Electrical conductance, $m\Omega^{-1}$ cm^{-1}	Nitrate -nitrogen found, p.p.m.
20	0·00	0·2	22·9
20	0·01	0·9	20·5
20	0·02	1·4	20·0
20	0·03	1·8	19·7
20	0·04	2·2	19·4
20	0·05	2·6	19·0
20	0·10	4·4	18·0

In some instances the ionic strength in a suspension of 5 g of soil in 100 ml of 0·02 N copper sulphate corresponded to 0·05 N copper sulphate, and then the potential decreased by about 5 per cent. But for such soils it is often possible to dilute the soil suspension to reduce the ionic strength. Increased anionic interference can also increase the potential and to some extent counteract the influence of the ionic strength. The interference from sulphate ions, however, is small; even the potential from 0·2 N copper sulphate was less than the potential corresponding to 0·5 p.p.m. of nitrate-nitrogen.

ANION INTERFERENCE—

The measured potential for a mixed anion solution is given by the equation

$$E = \text{constant} - 2{\cdot}3\frac{RT}{F}\log\left(A_{NO_3^-} + k_1 C_1^{1/n}\right),$$

where $A_{NO_3^-}$ is the nitrate-ion activity, k_1 is the selectivity constant for an interfering anion at a concentration C_1, and n the charge of the interfering anion.

Most anions occurring in soil have small selectivity constants but in saline soil, however, both Cl^- and HCO_3^- contribute to the measured potential, and the selectivity constant is 6×10^{-3} for Cl^- and 2×10^{-2} for HCO_3^-.

To evaluate the influence of HCO_3^- ions, we carried out some determinations of solutions that contained 10 p.p.m. of nitrate-nitrogen and varying amounts of sodium hydrogen carbonate. The results are shown in Table V.

TABLE V

NITRATE DETERMINATIONS AT DIFFERENT CONCENTRATIONS OF SODIUM HYDROGEN CARBONATE

Sample	Nitrate-nitrogen, p.p.m.	HCO_3^-, p.p.m.	Solvent	pH	Electrical conductance, $m\Omega^{-1}$ cm^{-1}	Nitrate-nitrogen found, p.p.m
1	10	0	Distilled water	6·9	0·1	10·0
2	10	100	Distilled water	7·9	0·2	10·1
3	10	200	Distilled water	8·2	0·3	10·1
4	10	500	Distilled water	8·4	0·7	10·0
5	10	1000	Distilled water	8·4	1·3	9·9
6	10	2000	Distilled water	8·4	2·4	9·4
7	10	5000	Distilled water	8·4	5·7	8·7
8	10	0	0·02 N Copper sulphate	4·8	1·3	10·0
9	10	100	0·02 N Copper sulphate	5·0	1·3	9·9
10	10	200	0·02 N Copper sulphate	5·2	1·3	10·1
11	10	500	0·02 N Copper sulphate	5·7	1·5	10·2
12	10	1000	0·02 N Copper sulphate	6·0	1·7	10·2
13	10	2000	0·02 N Copper sulphate	6·6	2·6	10·2
14	10	5000	0·02 N Copper sulphate	7·5	4·9	10·1

In distilled water the measured nitrate content is smaller than expected when the HCO_3^- concentration is increased. This is caused by the increased ionic strength, which influences the activity of the nitrate ion. Samples 1 to 7 contained only nitrate and varying amounts of sodium hydrogen carbonate, while samples 8 to 14 also contained copper sulphate corresponding to 0·02 N copper sulphate.

It seems to be advantageous to use 0·02 N copper sulphate instead of water as extracting agent. Relatively large amounts of sodium hydrogen carbonate do not significantly alter the values for the nitrate content in 0·02 N copper sulphate.

ACCURACY AND REPRODUCIBILITY—

As already shown, nitrate determinations with the nitrate electrode agree well with two colorimetric methods. In addition, recovery of added nitrate to soil suspensions was nearly 100 per cent. It is therefore probable that the results are reliable. However, if the nitrate-nitrogen concentration in the solution is below 2 p.p.m., the results differ slightly from those obtained by the other two methods. Between 0·5 and 2·0 p.p.m. the reproducibility was not good. There is interference from the relatively high ionic strength and also from some anions, but for concentrations above 2 p.p.m. the interferences do not seem to be serious.

To evaluate the reproducibility of the method, the coefficient of variation was calculated, both for filtered soil extracts and for soil suspensions. A 5-g sample of soil was extracted with 100 ml of 0·02 N copper sulphate. The coefficient of variation, as based on duplicate analyses of thirty-one soils for filtered extract soil, was 2·7 per cent., while for soil suspension the coefficient of variation was 2·0 per cent., as based on duplicate analyses of fifteen soils.

To obtain good results the electrode was checked at intervals. Below 2 p.p.m. of nitrate-nitrogen drift was troublesome, but readings at higher concentrations were steady.

CONCLUSION

Determination of nitrate in soil extracts and soil suspensions with the Orion nitrate electrode gives reproducible and reliable results. The nitrate determinations can be carried out in the same way as pH determinations, and this allows a rapid estimation of the nitrate status of soils. Interferences are not serious, and the coefficient of variation is about 2 per cent. It is possible to determine as little as 9 pounds of nitrate-nitrogen per acre, and possibly less. As the instrument is portable and equipped with a battery, rapid determinations can

be performed in the field as it is possible to measure the nitrate directly in a suspension after a few minutes of shaking with the extracting solution, therefore the method is useful for routine analyses of soils.

We thank Mrs. G. Aamodt and Mr. M. Monsen for technical assistance.

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Automated Distillation Procedure for the Determination of Nitrogen

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A distillation step has been incorporated into the automated determination of ammonia by the indophenol method.

By this means the application of the method has been extended, and interference from metal ions and the need for dialysis of turbid solutions have been eliminated. The reliability of the method has been improved without loss of speed.

NITROGEN is determined in a wide range of materials by destructive digestion, to convert the nitrogen into ammonium salts, and subsequent steam-distillation of ammonia into standard acid for final determination by back-titration. The introduction of the Technicon AutoAnalyzer system has resulted in the automation of the determination of such ammonium salts, without distillation, by using the reaction of ammonia with alkaline phenol and hypochlorite.[1] Although satisfactory for many purposes, the method has limitations that prevent its direct application to the wide range of conditions under which steam-distillation applies. The limitations arise from the sensitivity of the phenol - hypochlorite reaction to interference by several chemical species and to pH. Russell[2] found that the intensity of the blue colour, thought to be indophenol, produced by the reaction was strongly dependent on pH and was enhanced by iron, chromium and manganese(II) ions and inhibited by copper. Williams and Twine[3] had to guard against variations in the volume of sulphuric acid persisting in the final digests of plant material, but concluded that in such digests interference from metals was unlikely. However, Suzzarini[4] found that the AutoAnalyzer method gave slightly higher values than distillation for a range of plant samples. In order to eliminate these problems, which are more acute with materials such as animal faeces and soils, a distillation step has been introduced into the automated procedure.

EXPERIMENTAL

DISTILLATION—

The sample is mixed with 10 per cent. w/v sodium hydroxide and air, and is pumped through 2 feet of steel delay coil, immersed in a constant-temperature bath at 116° C. As the mixture emerges from the coil a stream of air is injected, which blows the mixture into a series of splash-heads. The trapped liquid is pumped to waste while the air stream containing steam and the ammonia passes through the splash-heads. A stream of 0·1 N hydrochloric acid is introduced into the air stream to absorb the ammonia, and the mixture passed down a condensing column to a phase-separator from which a sample is continuously withdrawn for the determination of ammonium-nitrogen. The configuration is shown in Fig. 1.

A simple constant-temperature bath was assembled in the laboratory and, because the design offers greater flexibility in research laboratories than the standard AutoAnalyzer bath, a brief description is given here. In addition to its use for the present nitrogen method, the same design of bath is used for other temperatures, and the description includes the standard-coil configurations for methods needing delay coils at temperatures below 100° C.

The tank of 20-s.w.g. stainless steel is 8 × 8 × 24 inches with a 1-inch flange. The lid, of $\frac{1}{2}$ inch thick laminated plastic (Tufnol), is held by bolts threading into a $\frac{3}{4} \times \frac{1}{2}$ inch thick mild-steel loose collar beneath the flange. A gasket of $\frac{1}{8}$-inch neoprene seals the joint. Heating and temperature control are provided by a Haake Unitherm with 1-kW external heater, giving 1500-W heating power. The unit is supported centrally in the bath. Glass coils in flat configurations are supported from $\frac{3}{16}$ inch thick brass cover plates, to which are also attached the cooling coils (Fig. 1). The bath has the capacity for a total of 280 feet of delay coil, and each unit of up to 40 feet with a cover-plate can be removed separately for servicing or modification. Glycerol is used in the heating-bath, as oil was found to fume after some time.

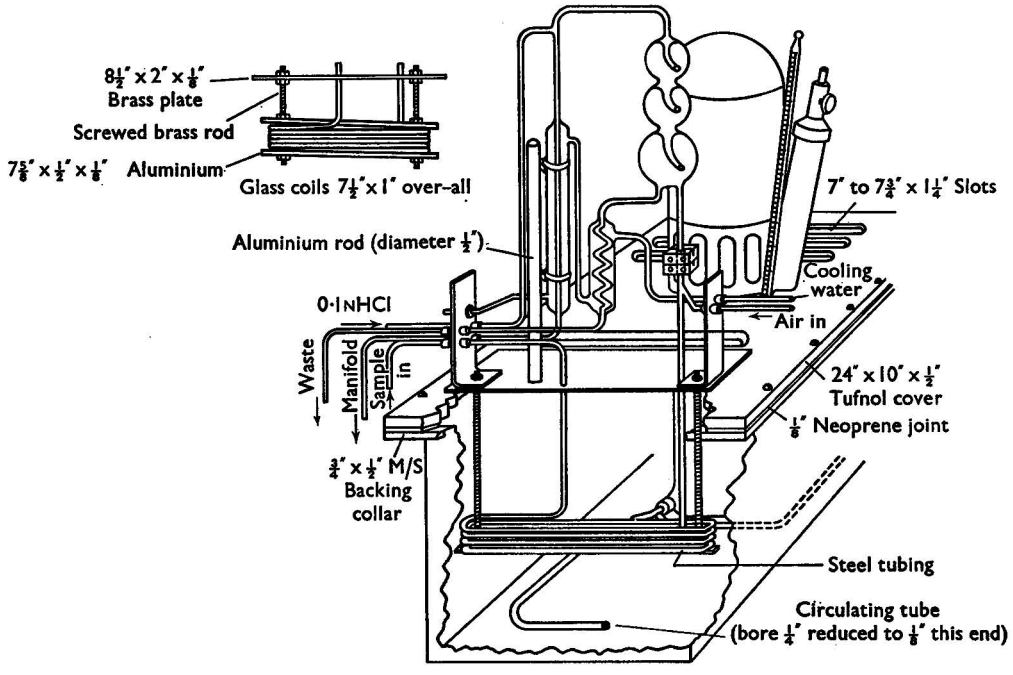

Fig. 1. The configuration of the distillation unit and its location in the constant-temperature bath

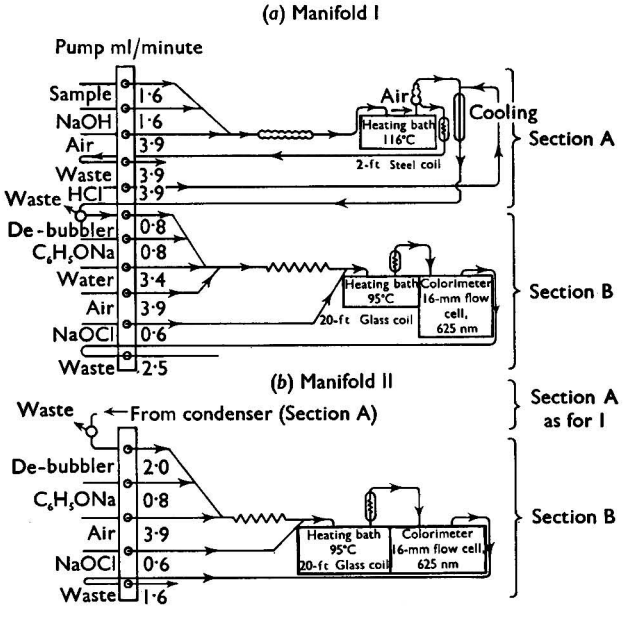

Fig. 2. The manifold and flow sequence for the determination of nitrogen in: (*a*) Manifold I, the range 0 to 200 mg of nitrogen litre^{-1}; and (*b*) Manifold II, the range 0 to 30 mg of nitrogen litre^{-1}

MANIFOLD CONFIGURATIONS—

The complete configuration of the manifold is shown in Fig. 2(*a*). As shown, and with the reagent concentrations described below, the method covers the range 0 to 200 mg of nitrogen litre^{-1}. For greater precision in the analysis of samples containing lower levels of nitrogen the alternative configuration in Fig. 2(*b*) can be used, which covers the range 0 to 30 mg of nitrogen litre^{-1}. Beer's law is obeyed over the complete range specified with both manifolds.

REAGENTS—

Alkaline phenate—Dissolve 200 g of phenol in about 100 ml of water. Dissolve 89 g of sodium hydroxide in 300 ml of water. When cool mix both solutions, make up to 1 litre and shake well. Store in a refrigerator.

For the range 0 to 30 mg of nitrogen litre^{-1}, use this solution and manifold II.

For the range 0 to 200 mg of nitrogen litre^{-1}, dilute 330 ml of this solution to 1 litre with water, and use manifold I.

Sodium hypochlorite—Dilute 250 ml of commercial sodium hypochlorite solution (12 per cent. of free chlorine) to 1 litre.

Standard nitrogen solutions—Dry ammonium sulphate for 2 hours at 105° C. Dissolve 4·716 g in water in a 1-litre calibrated flask and make up to the mark. This solution contains 1000 mg of nitrogen litre^{-1}. Prepare by dilution appropriate standards within the range 0 to 200 mg of nitrogen litre^{-1}.

Read the colour at 625 nm.

EFFECTS OF VARIABLES—

Rate of air flow—There was no effect of air flow on the chart reading within the range 3·8 to 14 ml s^{-1}. However, the higher rates of flow appeared to produce a noisier trace, and a rate of 4 ml s^{-1} was adopted for standard operation. No ammonia could be detected in the waste from the splash-head with this rate of flow.

Heating-coil configuration—Two main variations were tested, the length of coil preceding air injection and the length between injection and the splash-head. Increasing the length of coil preceding air injection from 2 to 15 feet had no effect. Introducing the air with 5 feet of coil within the bath before the splash-head resulted in high back-pressures on the input tubes and frequent breakage of the coils. No beneficial effect of the distance between injection and splash-head could be detected, and air is now injected about 3 inches beneath the splash-head (Fig. 1). As the hot alkaline solution attacks Pyrex glass, the heating coil is made of steel tubing. The connection between the splash-head and metal coil is made with a PVC sleeving tube held securely by split collars with screws.

Temperature of distillation—At 101° C the sensitivity was low and reproducibility poor. Increasing the temperature resulted in higher sensitivity and better reproducibility up to 116° C, after which any further increase in temperature reduced the sensitivity slightly (Fig. 3).

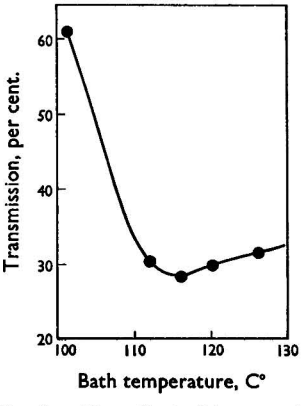

Fig. 3. The effect of temperature of the constant-temperature bath on the chart reading (transmission, per cent.)

Reagent concentrations—The effect of variables on colour development was examined by Russell,[2] and more recently by Tetlow and Wilson.[5]

A brief summary of the main effects is included. Highest sensitivity is generally achieved when phenol and sodium hydroxide are approximately equimolar.[2] With colour development at, or near, 100° C sensitivity can be readily changed by altering the phenol concentration within the range 1 to 8 per cent. in the final mixture. Higher pH values, as in the methods of Borsook,[6] and Williams and Twine,[3] result in less reproducible colour formation at, or near, 100° C. Lower temperatures at high pH values result in greater reproducibility but increase the time needed for the colour development.

When the final phenol concentration is in the range 1 to 8 per cent., the available chlorine should be in the range 0·15 to 0·25 per cent. w/v.[2] Varley[7] found the method satisfactory with about 16 per cent. w/v of phenol and about 2 per cent. of available chlorine in the final mixture.

Results

Accuracy—

The accuracy of the method was tested by comparison with manual distillation of a range of digests of plant samples. Samples of grass, wheat grain, pine needles and pine wood were digested with sulphuric acid and hydrogen peroxide. Several replicates of each sample were digested and aliquots of each resulting digest were supplied to three analysts in separate laboratories for manual distillation and titration, and were analysed on two separate days with the present automated method. The recorder charts obtained with the automated method were read by different assistants on the two days. All results were returned to a statistician without comparison by any of the analysts, and an analysis of variance was performed (Table I).

Table I

ANALYSIS OF VARIANCE OF RESULTS ON A SERIES OF PLANT DIGESTS BY MANUAL OPERATORS AND THE PRESENT AUTOMATED METHOD

			Operator using				
			Manual method			Automated procedure	
(*a*)	Sample	No. of experiments	1	2	3	4	5
	Grass	4	2·86	2·72	2·96	2·80	2·76
	Wheat grain	6	2·66	2·60	2·72	2·66	2·64
	Pine needles	6	0·44	0·42	0·46	0·44	0·48
	Pine wood and bark	6	0·11	0·10	0·13	0·10	0·12
	Pine needles	6	0·86	0·80	0·90	0·89	0·88
	Mean		1·28	1·23	1·33	1·27	1·28

	Source of variation	Degrees of freedom	Mean square	Variance ratio	
(*b*)	Samples	4	106685·37	5978·95*	
	Analysts	4	86·65	4·86†	
	Samples × analysts	16	15·90	1	N.S.
	Within groups	115	17·84		
	Total	139			
(*c*)	Comparisons—				
	(1 + 2 + 3) *vs* (4 + 5)	1		1	N.S.
	(1 + 2) *vs* (4 + 5)	1		1·80	N.S.
	(1 + 3) *vs* (4 + 5)	1		3·12	N.S.
	(2 + 3) *vs* (4 + 5)	1		1	N.S.
	(2 − 1) *vs* (5 − 4)	1		2·18	N.S.
	(3 − 1) *vs* (5 − 4)	1		2·67	N.S.
	(3 − 2) *vs* (5 − 4)	1		10·52	†

* Denotes significance at $P < 0·001$.
† Denotes significance at $P < 0·01$.
N.S. denotes not significant at $P = 0·05$.

As there was a significant effect arising from differences between analysts, a comparison was made of the mean results of the AutoAnalyzer runs and the mean results of manual analysis, paired in all combinations. No comparison was significant, indicating that the automated method introduced no bias in comparison with the manual method.

In addition, the difference between pairs of analysts was compared with the difference between the two automated runs. The difference between analysts 2 and 3 was significantly greater ($P < 0.01$) than that between the automated runs. Differences between laboratories were, therefore, greater than differences between runs in the automated method.

PRECISION—

The replication of samples in the test described above could not be used to compare precision of the methods as sampling and digestion errors were included. The precision of the method was examined by repeated determinations on a series of standard solutions and the results are given in Table II.

TABLE II

PRECISION OF REPEATED DETERMINATIONS ON THE SAME SOLUTIONS

Concentration, mg of nitrogen litre^{-1}	Standard deviation*	
	Manifold I	Manifold II
5	±0·06	—
10	±0·09	±0·32
20	±0·06	—
40	—	±0·48
100	—	±0·57

* Standard deviation of eight determinations calculated as $\sqrt{\frac{(x - \bar{x})^2}{7}}$.

The more sensitive manifold permits the determination of 5 mg of nitrogen litre^{-1} with a standard deviation of about 1 per cent.

For these tests a sample and wash time each of 60s was used.

APPLICATION—

The method permits the extension of the automated indophenol method to digests of soils and other materials with a high concentration of heavy metals. The use of a dialyser for turbid solutions can be dispensed with. As the ammonia in the present method is always in 0·1 N hydrochloric acid when introduced into the indophenol reaction, there is the added advantage that a single method can be applied without modification to digests of a wide range of materials, without the preparation of standards in separate supporting solutions to match the digesting conditions.

The speed of the determinations is not affected by the distillation, and greater confidence can be placed in the results from samples of unknown composition, as the presence of unsuspected ions that would bias the result from direct reaction with phenol - hypochlorite has no effect in the present procedure.

We are grateful to Messrs. M. A. Chapman, A. G. Turton, A. Hatch and T. C. Shaw for co-operation in manual analysis of samples, and N. A. Campbell for the statistical analysis, reported in Table I, and A. Wilkie for the drawing in Fig. 1, and the construction of the heating-bath.

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Dual Column and Derivative Techniques for Improved Specificity of Gas-Liquid Chromatographic Identification of Organochlorine Insecticide Residues in Soils

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Derivatives of organochlorine insecticides exhibiting R_t values substantially different from those of the parent insecticides were obtained for *pp'*-TDE, *pp'*-methoxychlor, *op'*-DDT and *pp'*-DDT on treatment with 2 per cent. ethanolic potassium hydroxide, and for endrin and dieldrin with ethanolic concentrated hydrochloric acid; partial clean-up of soil extracts was achieved by the alkali treatment. The use of dual columns, namely, DC-200 and diethylene glycol succinate, in conjunction with derivative formation, provided R_t values sufficiently different for improved organochlorine insecticide identification with minimal additional effort.

In contrast to most analytical methods, gas - liquid chromatography with the electron-capture detector has provided the analytical accuracy and sensitivity necessary for determining organochlorine insecticide content of soil samples. Unfortunately, the means of identifying unknown compounds is not based on a unique chemical characteristic but instead is based on the time (R_t value) required by the compound to traverse the column.[1] Consequently, identification of organochlorine insecticides in contaminated extracts based on R_t values obtained under a single set of conditions is not valid, and supplementary techniques are required to enhance the specificity of identification.

The use of dual gas - liquid chromatographic columns and formation of insecticidal derivatives that can be readily determined by gas - liquid chromatography has been suggested for the improved identification of insecticides.[2,3,4,5,6] These techniques are rapid and, with correct choice of columns or derivative preparation methods, provide different R_t values. A polar and a non-polar column, when paired, often provide markedly different R_t values for the improved probability of a correct identification.[6]

Derivative preparation techniques applied to biological extracts include de-hydrochlorination of DDT and its analogues,[3,4,5] *pp'*-methoxychlor[4] and the treatment of aldrin, dieldrin and endrin with hydrobromic acid.[3] These techniques have been evaluated on standard insecticide solutions,[3,4,5] vegetable[4] and animal-tissue extracts,[3,5] and the derivatives displayed R_t values substantially different from the parent compounds.

This investigation was initiated to obtain a rapid, effective method of improving identification of organochlorine insecticides in soil extracts with dual-column gas - liquid chromatography and derivative preparation techniques. In combination, these techniques provide four R_t values for each insecticide if a single derivative is formed.

Experimental

Standard insecticide solutions and "spiked" soil extracts containing γ-BHC, heptachlor, heptachlor epoxide, dieldrin, *pp'*-DDE, endrin, *pp'*-TDE, *op'*-DDT, *pp'*-DDT, *pp'*-methoxychlor and insecticide-contaminated soil extracts were subjected to dual-column gas - liquid chromatographic techniques and derivative preparation methods.

Instrumental—

A model 7620 Packard chromatograph, equipped with dual columns and tritium-foil electron-capture detectors, was used for the determination of organochlorine residue contents of soil extracts. Gas-chromatographic conditions were: glass columns, 4 mm i.d.; column, inlet and outlet temperatures, 200°, 235° and 225° C, respectively; detector sensitivity, 1×10^{-9} A full scale at 50 V and temperature 205° C; nitrogen was used as carrier gas at a flow-rate of 125 ml minute^{-1}.

Two types of column packing were used: 10 per cent. DC-200 on 60 to 80-mesh Gas Chrom Q (2 m × 4 mm i.d.), and 10 per cent. diethylene glycol succinate (DGS) on 60 to 80-mesh Gas Chrom Q (1 m × 4 mm i.d.). The column supports and coatings were obtained from Applied Science Laboratories Inc., P.O. Box 140, State College, Pennsylvania, U.S.A.

PROCEDURE—

Soils (100 g) were extracted with 200 ml of a hexane - acetone azeotropic mixture (41 + 59) on a Soxhlet extractor for 12 hours.[7] Acetone was removed from the extract with water (three washings, each of 50 ml) and the hexane layer was analysed by gas - liquid chromatography, or concentrated to 5 ml with a two-ball micro-Snyder column for derivative preparation.

Soil extracts and standards containing *pp'*-methoxychlor, *pp'*-DDT, *op'*-DDT or *pp'*-TDE were refluxed with 2 per cent. potassium hydroxide in 95 per cent. ethanol for 12 minutes at 80° C.[5] Extracts containing endrin and dieldrin were mixed with 5 ml of concentrated hydrochloric acid in 10 ml of 95 per cent. ethanol and heated at 80° C for 30 minutes. Hexane (20 ml) was added and the mixture partitioned with 100 ml of water to separate the hexane layer for gas - liquid chromatography. Because of the extremely short R_t value for aldrin on the DGS column, R_t values are expressed relative to heptachlor epoxide.

RESULTS AND DISCUSSION

Ethanolic potassium hydroxide or hydrochloric acid derivative methods were evaluated on standard insecticide solutions to establish R_t values for derivatives on the relatively polar DGS and non-polar DC-200 columns (Table I). The potassium hydroxide treatment applied to standard solutions and "spiked" soil extracts converted γ-BHC, *op'*-DDT, *pp'*-DDT, *pp'*-TDE and *pp'*-methoxychlor into their respective de-hydrochlorinated derivatives but did not degrade aldrin, heptachlor, heptachlor epoxide, dieldrin and endrin under the conditions used. The R_t values determined for each insecticidal derivative, except that for γ-BHC, were approximately one half of the R_t value of the parent insecticide determined on the DC-200 column; the derivative of γ-BHC was apparently eluted with the solvent. In addition, the potassium hydroxide treatment improved the chromatographic characteristics of soil extracts by removal of extraneous peaks and by base-line stabilisation.

TABLE I

R_t VALUES OF ORGANOCHLORINE INSECTICIDES AND THEIR CHEMICAL DERIVATIVES

Compound	R_t values relative to heptachlor epoxide on— DC-200	DGS
Insecticides—		
γBHC	0·38	0·70
Heptachlor	0·64	0·35
Aldrin	0·81	0·35
Heptachlor epoxide	1·00	1·00
pp'-DDE	1·48	1·23
Dieldrin	1·48	1·52
Endrin	1·65	1·72
pp'-TDE	1·96	3·92
op'-DDT	2·01	1·73
pp'-DDT	2·45	3·25
pp'-Methoxychlor	3·63	9·10
Potassium hydroxide derivatives of—		
op'-DDT	1·20	0·97
pp'-TDE	1·20	1·16
pp'-DDT (*i.e.*, *pp'*-DDE)	1·48	1·23
pp'-Methoxychlor	2·29	3·84
Hydrochloric acid derivatives of—		
Endrin	2·78	—
Dieldrin	2·90	—

Similarly, hydrochloric acid treatment resulted in complete conversion of endrin and partial conversion of dieldrin (about 50 per cent.) into derivatives with R_t values approximately twice those of the parent compounds, on the DC-200 column. Endrin and dieldrin derivative

peaks were not completely resolved, but were sufficiently separated for identification. The hydrochloric acid method had little effect on γ-BHC, aldrin, heptachlor and its epoxide, *pp'*-DDT and its analogues and *pp'*-methoxychlor. The hydrochloric acid derivatives were not characterised but were dissimilar apparently to hydrobromic acid derivatives; the relative R_t values for hydrobromic acid derivatives[3] are approximately twice those obtained with hydrochloric acid.

Although several combinations of insecticides were not resolvable on a single column, separate R_t values for all insecticides, except for *op'*-DDT, were obtained from the two columns (Table I). Interferences were observed between *op'*-DDT and either endrin on the DGS column or *pp'*-TDE on the DC-200 column. None of the fifty-one U.S. soils examined previously contained in excess of 10 μg per 1000 g of endrin, suggesting that the unresolved *op'*-DDT - endrin combination on the DGS column is not a serious limitation for survey investigations. However, separation is accomplished by treating the soil extract with either hydrochloric acid to remove endrin, or potassium hydroxide to convert *pp'*-TDE and *op'*-DDT into their derivatives, prior to analysis.

Following a survey of some fifty-one soil samples collected in nine American states,[8] several samples containing the most commonly occurring organochlorine insecticides were used to evaluate the applicability of the combined derivative and dual-column technique. Insecticides most commonly found were *pp'*-DDT and its analogues, dieldrin and γ-BHC. Typical chromatograms showing the results of the confirmatory tests on an acetone - hexane extract of a Longrie silt loam soil sample from Wisconsin are shown in Figs. 1 and 2. Insecticidal concentrations (μg per 1000 g) in the Longrie extracts are: dieldrin, 340; *pp'*-DDE, 500; *op'*-DDT, 200; *pp'*-TDE, 60; and *pp'*-DDT, 3800. These insecticides, except for *pp'*-TDE (about 30 μg per 1000 g), could be determined in soil extracts at one fifth of the aforementioned concentrations. The potassium hydroxide derivatives were determined at sensitivities similar to those for the parent compounds; hydrochloric acid derivatives of dieldrin and endrin required minimal concentrations of 100 μg per 1000 g.

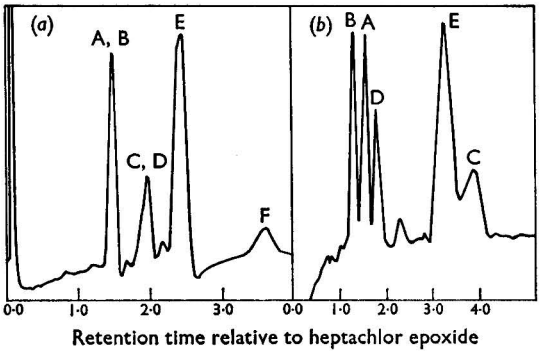

Fig. 1. Identification and confirmation of insecticide residues in untreated extracts of Longrie silt loam by gas - liquid chromatography on (*a*), DC–200, and (*b*) DGS columns. A, dieldrin; B, *pp'*-DDE; C, *pp'*-TDE; D, *op'*-DDT; E, *pp'*-DDT; and F, *pp'*-methoxychlor

Although the DC-200 column does not differentiate between dieldrin and *pp'*-DDE, and *pp'*-TDE and *op'*-DDT [Fig. 1 (*a*)], these compounds are resolved on the DGS column [Fig. 1 (*b*)]. The presence of dieldrin is confirmed from its hydrochloric acid derivative on the DC-200 column [Fig. 2 (*a*)]. The confirmation of *pp'*-DDT is strengthened by reinforcement of the *pp'*-DDE *plus* dieldrin peak on the DC-200 column [Fig. 2 (*b*)] and a confirmatory R_t value for *op'*-DDT was obtained by chromatographing the potassium hydroxide derivative on the DGS column [Fig. 2 (*c*)]. The appearance of a peak corresponding to the R_t value of *pp'*-methoxychlor on the DC-200 column was not confirmed by subsequent examination of the soil extract on the DGS column or after potassium hydroxide treatment. However, the presence of *pp'*-TDE, apart from its appearance on the DGS column, cannot be confirmed.

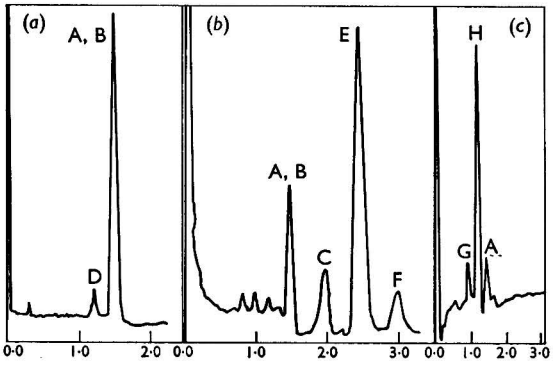

Fig. 2. Identification and confirmation of insecticide residues in Longrie silt loam by gas - liquid chromatography (*a*), ethanolic potassium hydroxide derivatives on DC–200; (*b*), ethanolic hydrochloric acid derivatives on DC–200; and (*c*) ethanolic potassium hydroxide derivatives on DGS. A, dieldrin; B, *pp'*-DDE; C, *pp'*- TDE or *op'*-DDT,or both; D, potassium hydroxide derivatives of *op'*-DDT or *pp'*-TDE, or both; E, *pp'*-DDT; F, hydrochloric acid derivative of dieldrin; G, potassium hydroxide derivative of *op'*-DDT; and H, *pp'*-DDE or the potassium hydroxide derivative of *pp'*-TDE, or both

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The Spectrophotometric Determination of Riboflavine in Urine

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A simple spectrophotometric method for the determination of riboflavine in urine is described. The urine, after treatment with zinc acetate and formaldehyde, is passed through a small column of large particle-size talc on which the riboflavine is selectively and quantitatively adsorbed. Successive washings with 0·01 N hydrochloric acid and 5 per cent. dioxan remove the urinary pigments and other urinary constituents almost completely. The riboflavine eluted with 20 per cent. dioxan is measured spectrophotometrically at 444 nm. Some results are given for the rate of excretion of riboflavine in urine following intravenous injection of the vitamin.

As much of what is known of the metabolism of riboflavine is based on studies of its excretion in urine and as riboflavine deficiency is frequently encountered in man, it is important to evolve a rapid, accurate and specific diagnostic method for the determination of riboflavine in urine. For this purpose chemical methods of determination are clearly to be preferred to the more laborious and time-consuming biological and microbiological methods.[1,2]

The fluorimetric methods widely used for the determination of riboflavine in urine and other solutions, which form the basis of the U.S. Pharmacopoeia method, are non-specific, as reported by Morell and Slatter,[1] who compared seven different methods for determining riboflavine fluorimetrically in urine and modified that used by Najjar.[2] With these methods interference from fluorescent substances other than riboflavine cannot be excluded. Either chemical treatment alone or its combination with chromatographic separation is necessary to eliminate the effects of other substances,[3,4,5] and comparison with standard riboflavine solutions, similarly treated, is also necessary to show the effect of chemical treatment on the vitamin. Florisil adsorbed riboflavine readily, but elution was incomplete.[1,6]

Even the specificity of the microbiological method cannot be accepted without question, as reported by Price.[7]

A spectrophotometric method has been described for the determination of riboflavine in simple solutions and tablets and was found to be specific, accurate and simple.[8] The full spectrum of riboflavine in pH 4·0 buffer has three characteristic peaks at 267, 375 and 444 nm, and the ratios of extinctions at 375 and 444 nm to the extinction at 267 nm should lie within the limits of 0·314 to 0·333 and 0·364 to 0·388, respectively, as stated in the British Pharmacopoeia. In more complex formulations, interfering substances are eliminated either by physical or chemical processes, or both.[9]

The spectrophotometric method can also be used successfully for the quantitative separation of riboflavine from vitamin mixtures.[10]

With urine, it is necessary to eliminate the effects of urinary pigments and other constituents that may interfere with the light-absorbing properties of riboflavine. In the method described below, successful separation of the riboflavine from urine is achieved following its selective adsorption on a column of talc of a special particle size. The riboflavine is then quantitatively eluted in a sufficiently pure state to permit observation of its full absorption spectrum.

Method

Apparatus—

A Zeiss spectrophotometer, or equivalent, and a column, 12 × 200 mm, with stopcock, connected to a suction pump through the receiver, were used.

REAGENTS—

Zinc acetate.

Formaldehyde solution (formalin, 40 per cent.).

Hydrochloric acid, 0·01 N.

Talc, acid purified—Obtainable from British Drug Houses Ltd.

Dioxan—Analytical-reagent grade. This was checked to ensure that its absorbance at 267, 375 and 444 nm did not exceed 0·03. Lower grades of this reagent can sometimes be effectively purified by charcoal treatment.

PREPARATION OF TALC COLUMN—

The use of talc of a special particle size is necessary and this is prepared as follows. Suspend 100 g of talc in a large volume of water in a 1-litre Erlenmeyer flask, and allow it to settle. Decant the unsettled portion to waste after 3 to 5 minutes. Repeat this operation several times until the supernatant water no longer shows turbidity or opalescence. Pour a slurry of the talc into the column sufficient to reach a height of about 80 mm, with a small cotton-wool pledget at the bottom. Apply gentle suction to give a flow-rate of about 80 to 100 drops (4 to 6 ml) minute^{-1} and wash with water until the washings are clear.

The column is capable of several operations, provided it is adequately washed after each operation with water followed by about 30 ml each of 20 per cent. ethanol and water.

EFFECT OF PARTICLE SIZE OF TALC—

The particle size of the talc is very important as talc is ordinarily considered to be one of the substances on which riboflavine is not adsorbed (Sebrell and Harris[11]). Very fine talc has been shown to have little retentive power. The band of riboflavine adsorbed on ordinary talc was broad and readily eluted, and the spectrophotometric absorption of the eluate showed the presence of much extraneous matter. On coarse talc, prepared as described above, the riboflavine band was narrower and less readily eluted, and the eluate gave absorption curves typical for this vitamin. For accuracy, reproducibility and to ensure a reasonable flow-rate, it was necessary to remove all of the fine powder. A sample of talc, after treatment as described above, gave particles of between 20 and 50 μm diameter.

PROCEDURE—

Carry out the operation in a diffused light, avoiding direct sunlight; if solutions need to be left to stand for any length of time, keep them in darkness.

Collect freshly voided urine in glass vessels and store it in amber-glass bottles. After measuring the volume add 100 mg of oxalic acid for each 25 ml, to ensure that the pH is between 3 and 6, at which level of acidity riboflavine is maximally stable.

Treat the urine with zinc acetate (2 g per 100 ml of urine) and formaldehyde solution (formalin, 40 per cent.) (2 ml per 100 ml of urine), leave for half an hour and filter through paper known not to adsorb riboflavine. The zinc acetate eliminates the suspended particles,[12] which interfere with the adsorption, while the addition of formaldehyde[13] causes changes to occur in the chromogens usually present in urine that enable them to be readily washed from the column. Pass 100 ml of the filtered urine through the talc column at the rate of about 4 to 6 ml minute^{-1}. Wash the column walls with a little water, then pass through the column 20 ml of 0·01 N hydrochloric acid followed by 20 ml of 5 per cent. dioxan to elute the pigments and other urinary constituents. The riboflavine will be adsorbed on the top of the column. Add 20 per cent. dioxan until the yellow band approaches the bottom of the column. When it reaches the stopcock, collect the effluent until all of the yellow colour has passed through the stem. Measure the volume of the eluate, add 2 ml of 0·01 N hydrochloric acid and dilute to 25 ml with water. Prepare a blank of the same volume of 20 per cent. dioxan diluted similarly to 25 ml. Measure the maximum absorbance at 444 nm and calculate the result by using $E^{1\%}_{1cm} = 320$. Confirm the result and identity of the riboflavine by measuring the absorbance at 375 and 267 nm.

FULL SPECTRUM OF THE RIBOFLAVINE SEPARATED FROM URINE—

Riboflavine (1 mg) was added to 100 ml of normal fresh urine and the urine then treated as described above. The full spectrum for the urine eluate, after removal of pigments and other urinary constituents by adsorption on talc, is shown in Fig. 1. This procedure was repeated several times by using different samples on different days.

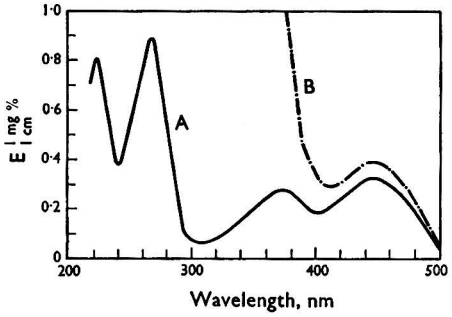

Fig. 1. A, typical riboflavine spectrum after removal of urinary pigments and other urinary constituents by adsorption on talc; B, spectrum of urine before adsorption on talc

A similar experiment was carried out on a pure solution of riboflavine (100 ml of solution containing 1 mg of riboflavine) and the spectrum compared with that of the eluate from urine. The spectra were found to be identical.

The full spectrum of the eluate from urine shows the three characteristic peaks of pure riboflavine at 267, 375 and 444 nm, and the ratios of extinctions at 375 and 444 nm to that at 267 nm, were found to be 0·314 and 0·370, respectively, which are within the limits given by the B.P. (0·314 to 0·333 and 0·364 to 0·388, respectively).

It is thus established that only riboflavine is measured by this procedure.

IDENTIFICATION OF RIBOFLAVINE IN THE ELUATE—

Passage through the talc column of 20 ml of 0·01 N hydrochloric acid followed by 20 ml of 5 per cent. dioxan was sufficient to elute all of the urinary pigments, as the last 5 ml of dioxan were colourless in all of the samples analysed.

The physical properties of the 20 per cent. dioxan eluate, the riboflavine content of which is to be determined, satisfied the requirements of the B.P. 1963 (p. 711). It had a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence, which disappeared on the addition of acids, alkalis or reducing agents such as dithionite solution (sodium hydrosulphite).

Reduction of riboflavine with dithionite has been used as the basis of a difference fluorimetric or spectrophotometric method for the determination of riboflavine in pharmaceutical products.[9] By this reduction riboflavine is converted into its leuco form (dihydroriboflavine), which is non-fluorescent in the visible region. On aeration of this solution in diffused daylight at room temperature for 1 minute, the fluorescence re-appears with the same intensity and has the same spectrum as pure riboflavine.

Riboflavine is determined in the eluate by the proposed method. The extinction at 444 nm is measured after addition of 0·1 ml of 5 per cent. dithionite solution (freshly prepared in water) to 25 ml of the eluate and allowing to stand for 1 minute, and again after aeration of the dithionite-treated solution for 1 minute. The difference between the two readings is compared with that obtained for pure riboflavine under the same conditions, and the riboflavine content calculated by using a value of 264 for the difference in $E^{1\%}_{1cm}$ at 444 nm between riboflavine and the leuco form.[9]

The results correlate with those of the proposed method. The full spectrum was also observed for the eluate after aeration and found to be similar to that for the original eluate.

Any other pigment present in the eluate would be reduced by the dithionite but not re-oxidised by aeration.[9] Thus there will be a difference between the intensity of the fluorescence or the extinction at 444 nm before and after aeration, which has not been found in any of the samples of urine analysed.

This establishes that the eluate contains only riboflavine and that the proposed method is selective for the determination of riboflavine in urine.

Results

Reproducibility of results and recovery from normal urine—

The present method yields reproducible results, and recovery is satisfactory when riboflavine is added to urine, as shown in Table I. The values given are the means of duplicate determinations and are representative of the many analyses that have been carried out.

Table I

Recovery of riboflavine from normal urine

Urine sample	Amount of riboflavine added, μg per 100 ml	Amount of riboflavine recovered from urine, μg per 100 ml	Difference
A	250	242	− 8
	500	510	+10
B	350	343	− 7
	650	635	−15
C	800	780	−20
	1000	1010	+10
D	400	390	−10
	700	700	0
E	450	435	−15
	900	880	−20

Mean difference −7·5. Standard deviation ±11.
Standard error of difference ±3·48.

Application of the *t*-test at degree of freedom 9 and P = 0·05 and 0·01 has shown that the difference between the amount added and that recovered is insignificant as calculated *t* equals 2·15.

Therefore, by the proposed method the amount recovered is almost exactly equal to the amount added, the difference observed being caused by experimental error.

Application of the method—

The smallest concentration that can be accurately determined by this method is about 0·20 mg of riboflavine per 100 ml of urine.

Tests in which a standard dose is used are more satisfactory than a determination made on a 24-hour specimen, because the daily excretion is greatly influenced by the amount consumed in the diet.[14,15] Sometimes, however, the amount of riboflavine present in normal urine is too minute to be adsorbed and quantitatively eluted from talc.

This method is useful in following the disappearance curve of riboflavine after intravenous injection; 16 μg of riboflavine per kg of body weight are given intravenously, and the amount of riboflavine excreted during the following 24 hours is determined. Results obtained are shown in Table II. The values given are the means of duplicate determinations and are representative of the many analyses that have been carried out.

Table II

Rate of excretion in urine of intravenously administered riboflavine

Case	Riboflavine given by intravenous injection, μg	Riboflavine excreted in the urine within the stated time intervals (in hours) following injection, μg				
		(1)	(2)	(4)	(6)	(24)
I	1500	560	580	590	605	630
II	1300	490	510	530	545	575
III	1100	415	435	450	460	480
IV	1000	380	400	415	425	435

Of the administered riboflavine 37 to 38 per cent. was excreted in the urine within 1 hour after injection, 39 to 40 per cent. after 2 to 4 hours, 41 to 42·5 per cent. after 6 hours and 42 to 44 per cent. after 24 hours. These results are similar to those obtained by Axelrod, Spies, Elvehjem and Axelrod.[16]

Discussion

The spectrophotometric method for the determination of riboflavine is found to be specific, accurate and simple. So far, no simple procedure was available capable of eliminating the urinary pigments and other constituents, which interfere with the light-absorbing properties of riboflavine, to allow direct spectrophotometric measurement. Separation on talc as described above makes this possible. This is because talc, being one of the weakest adsorbents, allows the urinary pigments and other constituents of urine to pass freely, yet it has a sufficiently high affinity for riboflavine to provide a specific means for its determination. The method combines the advantages of a simple chromatographic separation with the accuracy of spectrophotometry, and is more selective and flexible than fluorimetric procedures.

It is not necessary to check the results against a standard solution, nor to compare them with those of microbiological assays, because the method enables the full spectrum of the separated riboflavine to be observed, with its characteristic multiple peaks. Also, the ratios of extinctions lie within the limits stated by the B.P. and are identical with those of pure riboflavine. Indeed, the method includes its own means of identifying the riboflavine and confirming its purity.

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The Analysis of Mixtures of Methyl Ethanesulphonate and Ethyl Methanesulphonate by a Differential Reaction Rate Method

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Mixtures of methyl ethanesulphonate and ethyl methanesulphonate were determined by the differential reaction rate method. Suitable reaction rates for the analysis were given by the solvolysis of the mixture in butanol at 100° C.

During an investigation of the mechanism of the isomerisation of dimethyl sulphite to methyl methanesulphonate, it was necessary to analyse mixtures of methyl methanesulphonate, ethyl methanesulphonate (EMS), methyl ethanesulphonate (MES) and ethyl ethanesulphonate.

The mixture was separated, by gas - liquid chromatography, into three fractions, the second of which was a mixture of MES and EMS. This fraction was trapped from a preparative column, but attempts to separate the two components on several different columns were unsuccessful.

The differential reaction rate method for the analysis of binary mixtures[1,2] was therefore tested and found to be suitable for application to the fraction containing a mixture of EMS and MES. In this method, the components of the mixture react with a common reagent at different rates. If the over-all reaction rate is measured, equations can be formulated relating the rate of reaction and the initial concentrations of the components of the mixture.

The rates of several solvolytic reactions of MES and EMS were measured to find the optimum conditions for the analysis. The most suitable reaction was the solvolysis of MES and EMS in butanol at 100° C.

Experimental

Reagents—

Analytical-reagent grade butanol was used without further purification. Sodium butoxide (0·1 N) in butanol solution, prepared by dissolving sodium in butanol, was standardised with 0·1 N hydrochloric acid.

EMS prepared by the reaction of methanesulphonyl chloride with sodium ethoxide[3] was distilled, then re-distilled under reduced pressure, b.p. 87° C at 5 mm pressure of mercury. MES was similarly prepared from ethanesulphonyl chloride and sodium methoxide, b.p. 78° C at 6 mm pressure of mercury.

Procedure—

The reaction rates were followed by continuous automatic potentiometric titration of the alkanesulphonic acids as they were liberated. The titrant was 0·1 N sodium butoxide in butanol. A Pye Dynacap pH meter and Pye auto-titrator with an E.I.L. glass electrode (G.H.S. 23) and Cambridge calomel electrode with sintered-glass plug were used. The glass electrode was stored in N hydrochloric acid, otherwise, with continuous use in butanol, the response became sluggish.

The reaction was carried out in a 500-ml stirred reaction vessel, placed in an oil-bath maintained at 100° ± 0·05° C by a thermostat.

Results

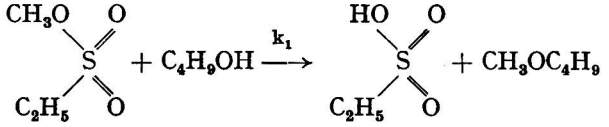
The rates of solvolysis of MES and EMS in butanol were measured separately, and the results are shown in Table I. Both reactions were pseudo first-order, as butanol was present in large excess.

TABLE I

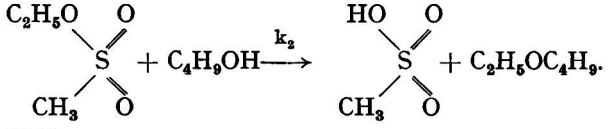
RATES OF SOLVOLYSIS OF MES AND EMS IN BUTANOL

	Reaction rate $\times 10^4$ s^{-1}	Temperature, °C
MES	2·52 ± 0·01	98·6
EMS	1·07 ± 0·02	98·3

The over-all reaction is given by the following equations—



MES



EMS

The rate equations for these reactions are—

$$[\text{MES}] = [\text{MES}]_0\, e^{-k_1^* t} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

and

$$[\text{EMS}] = [\text{EMS}]_0\, e^{-k_2^* t} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where $[\text{MES}]_0$ and $[\text{EMS}]_0$ are the initial concentrations of MES and EMS, $k_1^* = k_1[C_4H_9OH]$ and $k_2^* = k_2[C_4H_9OH]$.

If $[X]$ is the concentration of alkanesulphonic acid at any time t, then

$$[X] = [\text{MES}]_0 - [\text{MES}] + [\text{EMS}]_0 - [\text{EMS}] \quad .. \quad .. \quad .. \quad (3).$$

If a_0 is the initial concentration of MES and EMS, ($a_0 = [\text{EMS}]_0 + [\text{MES}]_0$), then by substituting equations (1) and (2) in equation (3), we have

$$[X] = a_0 - [\text{MES}]_0\, e^{-k_1^* t} - [\text{EMS}]_0\, e^{-k_2^* t}.$$

Therefore, $\log_{10}(a_0 - [X]) = \log_{10}\{[\text{MES}]_0\, e^{-k_1^* t} + [\text{EMS}]_0\, e^{-k_2^* t}\}$.

If $\log_{10}(a_0 - [X])$ is plotted against t, in general a curve will be obtained. However, when the concentration of the faster reacting component (MES) becomes negligible, then

$$\log_{10}(a_0 - [X]) = \log_{10}[\text{EMS}]_0 - \frac{k_2^* t}{2{\cdot}303}.$$

Under these conditions, *i.e.*, when all the MES has reacted, a plot of $\log_{10}(a_0 - [X])$ against t gives a straight line of slope $-k_2^*/2{\cdot}303$ and intercept $\log_{10}[\text{EMS}]_0$. $[\text{MES}]_0$ is found by subtracting $[\text{EMS}]_0$ from a_0.

The result of a typical experiment with a mixture of MES and EMS is shown in Fig. 1.

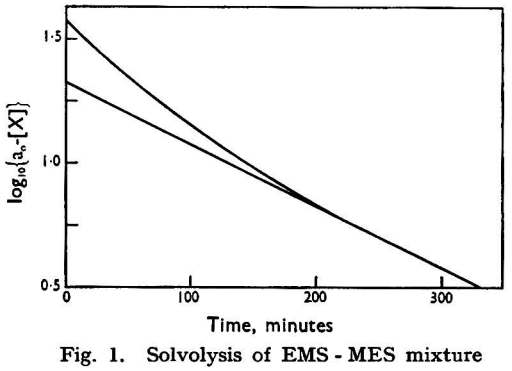


Fig. 1. Solvolysis of EMS - MES mixture

Table II shows the results of the analysis of a mixture of MES and EMS carried out in duplicate.

TABLE II

ANALYSIS OF MIXTURES OF EMS AND MES

	Added, per cent.	Found, per cent.
MES	43·4	42·7, 44·7
EMS	56·6	57·3, 55·3

A least-squares treatment of the points on the linear part of the curves shows that the accuracy of the method is about ± 3 per cent. at this ratio of the components of the mixture.

For mixtures of EMS and MES k_1/k_2 is 2·35. Greinke and Mark[4] have shown that for reliable results when k_1/k_2 is 2·35, the percentage of the faster-reacting component of the mixture (MES) must not exceed 45. The accuracy of the method increases as the percentage of MES decreases.

METHOD

Fill the reaction vessel with 500 ml of butanol and fit the lid, together with the electrodes, stirrer, thermometer and burette, filled with 0·1 N sodium butoxide solution, on to the vessel. Place the vessel in the oil-bath maintained at 100° C and start the stirrer. Allow 30 minutes for the vessel to attain a steady temperature, then connect the electrodes to the pH meter, set on the 400 to 600-mV range. Set the auto-titrator controller.

Note the burette reading, R_0. From a weighing pipette, add between 0·3 and 0·5 g of the MES - EMS mixture to the reaction vessel and start the clock. Record the burette reading, R_x, every 20 minutes and the corresponding time, t_x. Allow the reaction to proceed for 6 hours.

Calculate the final titre, a_0, from the weight of sample taken—

$$a_0 = \frac{\text{Sample weight}}{124} \times 10{,}000.$$

Plot log $(a_0 - X)$ against time t_x, where $X = R_x - R_0$.

The final part of the graph is a straight line. Produce this line back to find the intercept, I_E.

Then

$$\text{EMS, per cent.} = 100 \times \frac{\text{antilog } I_E}{a_0}$$

$$\text{MES, per cent.} = 100 - \text{EMS, per cent.}$$

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A Simple Trace Reader with Digital Print-out

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The construction and performance of an inexpensive, manually operated, electromechanical trace reader is described. Function generator circuits involving the use of biased diode networks are used to simulate convex and sigmoid calibration graphs by a series of linear approximations. The outputs of these circuits are used to drive a digital voltmeter with print-out. Comparative trials of the conventional manual procedure and the trace reader are reported. A measurement rate of 30 peaks minute^{-1} and precision of 0·5 per cent. f.s.d. are readily achieved with the trace reader. The system is less demanding on the powers of concentration of the operator, reduces the risk of transcription error and doubles the measurement rate of a single operator.

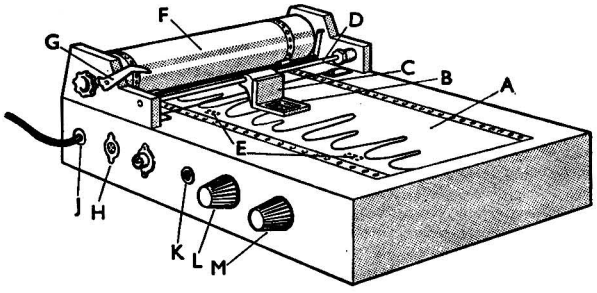
The results of an increasing number of analyses in chemical pathology and other analytical laboratories are being produced automatically and recorded as peaks on a chart. The amplitude of these peaks is subsequently measured, and the unknown concentration deduced by comparison with a calibration graph. This manual procedure is simple but tedious when many results are to be computed. Operator fatigue can be substantially reduced by the use of either a manually operated electromechanical trace reader or a fully automatic data-handling system. The latter solution is complex and costly, and justifiable only when large numbers of identical analyses are required. On the other hand, manually operated trace readers, in which the operator indicates the position of a peak by means of a pointer and a simple function generating circuit produces a voltage that varies linearly with concentration, are flexible and relatively inexpensive. Commercial trace readers are generally more elaborate and more accurate than necessary for the measurement of recordings from automatic analytical apparatus when a precision of 0·5 per cent. of f.s.d. is generally adequate.

The trace reader described in this paper* was constructed to meet the need for an inexpensive instrument of moderate precision for the rapid measurement of Technicon Auto-Analyzer chart records. With this trace reader a measurement rate of 30 peaks minute^{-1}, with a reproducibility (coefficient of variation) of better than 0·5 per cent. of f.s.d., is achieved.

Construction of the Instrument

The instrument is shown in Fig. 1. The chart is placed on the bed of the instrument, and peak heights on the chart are translated into voltage via a precision potentiometer. Rotation of the wiper arm of the potentiometer is produced by a wire fastened to a carriage, which runs on a track above the chart. The graticule attached to the carriage is manually aligned on the peak to be measured, and the output voltage of the potentiometer corresponding to the peak height is fed to a function generating circuit (Fig. 2). This circuit produces a voltage that is digitally equal to the concentration of the element or compound generating the peak. The calibration graph of the analysis (Fig. 3) is simulated by the summation of a number of linear functions. The voltage output is measured by a digital voltmeter (D.V.M.) (Dynamco DM2001 MK2), which drives a printer (Addo MOD11–0141); alternatively, a tape punch could be used. The printer command is by a press button on the chart-recorder chassis or by a foot control.

* Available commercially from Chemical Electronics Ltd., Birtley, Co. Durham.



A = Chart
B = Graticule
C = Carriage
D = Track
E = Base-line indicator light
G = Paper-clamping lever
H = Output to function generating circuit
J = Power supply
K = Printer command button
L = Set zero potentiometer
M = Set sensitivity potentiometer

Fig. 1. Details of the trace reader

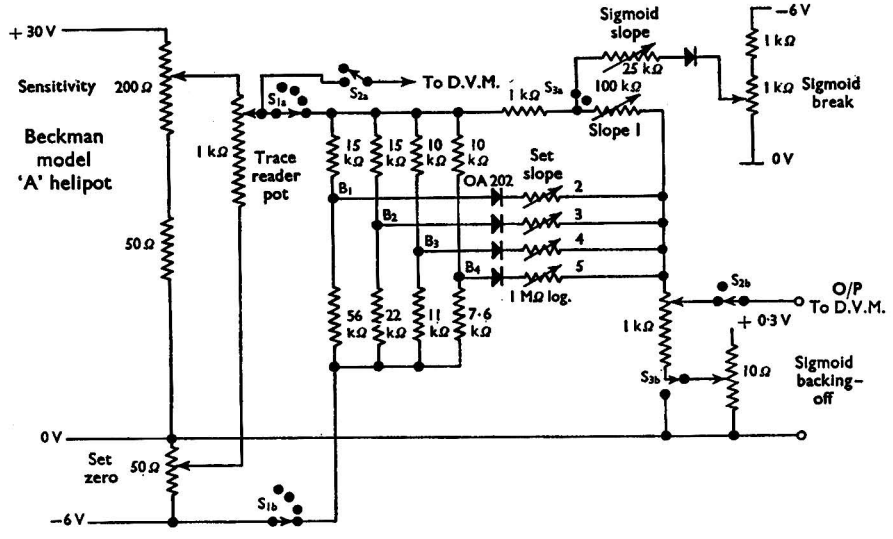


Fig. 2. Circuit diagram of function generating network

The function generating circuit is a conventional biased diode network with provision for accommodating changes in the base-line and sensitivity of the chart record. The break points of the calibration graph, B_1, B_2, B_3 and B_4 in Fig. 3, are pre-determined by the bias applied to the diodes and correspond to 20, 40, 60 and 80 per cent. of the full-scale reading. The circuit is "programmed" by adjusting the series resistances of the diodes to produce the required "slope" of the graph. The calibration procedure for a circuit selected by switch S1a,b is as follows.

1. A calibration graph of peak height, read manually, against concentration is plotted and the break points marked on the graph. The best linear approximation to each segment of the smooth graph is then drawn.

2. A piece of chart paper, graduated in the units used for the manually read calibration graph, is mounted on the reader desk.

3. The reader graticule is set to the base-line of the chart and the reading of the D.V.M. adjusted to read zero by using the "set zero" potentiometer.

4. The graticule is then aligned on the peak reading of the top standard, the D.V.M. is switched to the Standardise position, S2a, and the "sensitivity" potentiometer adjusted until the D.V.M. reads 15 V. The D.V.M. is now switched to its Operate position, S2b, with the diode series resistors set to mid-position. The "output" potentiometer is then adjusted until the D.V.M. reading is digitally equal to the concentration of the top standard.

5. Finally, the graticule is aligned in turn on the peak readings corresponding to the points at which the calibration graph and its linear approximation intersect, and the appropriate diode series resistor is adjusted until the D.V.M. gives the correct reading in concentration units. Following these settings it may be necessary to re-adjust the output potentiometer to accommodate the top standard; if so, the settings for the intermediate standards may then need slight modification.

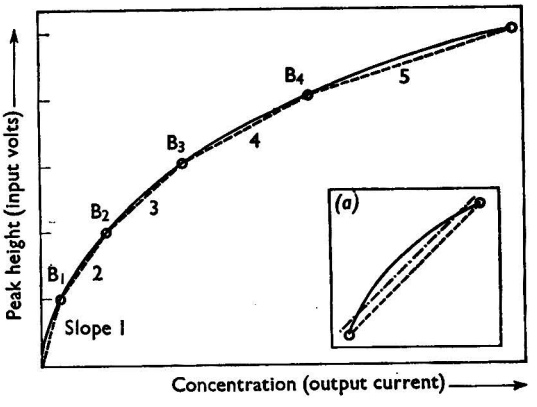


Fig. 3. Hypothetical calibration graphs. (*a*) Enlarged section of calibration graph showing improved accuracy of race-reader approximation: ⊙ calibration points; —— "true" calibration graph; ----- manual approximation; and —.—. trace-reader approximation

It has been found that the calibration graphs are constant for long periods and require only adjustment of the sensitivity potentiometer to accommodate day-to-day changes. Several function generating circuits, any one of which can be selected by a switch, have been programmed with frequently required calibration graphs. A sigmoid calibration graph can be accommodated by the addition of a biased diode in parallel with the output potentiometer, S3a, while a closer fit to the experimental graph can be obtained by the addition of more biased diodes providing intermediate break points.

Operation of the Instrument

The recorder chart is drawn through the instrument by means of the paper feed roller from a typewriter. Small linear drift in the base-line of the recording can be corrected by drawing a linear base-line on the chart and aligning this with the base-line indicator slits[1] of the instrument before clamping the paper with the feed roller. Larger changes in base-line and sensitivity can be compensated for by adjustment of backing-off and driving voltages in the function generating circuit.

When a chart is to be measured, first the appropriate function generating circuit is selected, S1a,b, and the base-line aligned. Next the graticule is aligned on a peak corresponding to a solution of known concentration, and the sensitivity control adjusted until the reading of the digital voltmeter is numerically equal to the known concentration. Unknown solutions are measured by aligning the graticule on their corresponding peaks; the concentration will then be displayed on the D.V.M.

Trials of the instrument

These were designed to give an objective assessment of the trace-reader's performance in comparison with that achieved by the manual procedure previously followed.

For convenience, the analytical method chosen to provide chart records for measurement was the determination of serum calcium with the Technicon AutoAnalyzer.[2] First, several standard solutions were analysed to check the validity of the calibration graph produced by the function generating circuit. The results were satisfactory and are shown in Table I.

Table I

Test of the validity of the calcium calibration graph

Concentration of standards, mg per 100 ml	2·50	5·00	7·50	10·00	12·50	15·00
Observed concentration, mg per 100 ml .. (Mean of six observations)	2·48	4·98	7·55	9·98	12·53	14·99
Standard deviation	0·04	0·04	0·04	0·02	0·07	0·09

The trace reader was set up with the reading of the 10 mg per 100 ml standard.

A number of peaks of varying amplitude was then measured in triplicate by both the manual and trace-reader methods. The mean values are presented in Table II. These results show a significant systematic difference ($t > 0{\cdot}1$ per cent. significance level) between the two methods of 0·1 mg per 100 ml, which can be attributed to the difference in the calibration graphs used (Fig. 3). In the manual method the calibration graph is obtained by joining calibration points with straight lines, thus solutions of intermediate concentration will always appear to contain more than their true concentration. The trace-reader calibration procedure produces equal positive and negative errors. The dead zone of the trace reader was found to correspond to 0·02 mg per 100 ml.

Table II

Comparison of results of calcium analyses measured with the manual method and the electronic trace reader

										Mean
Manual .. .. (mg per 100 ml) (Mean of three observations)	2·83	5·10	5·47	7·53	8·20	9·40	10·03	10·43	10·90	13·77	8·366
Trace reader .. (mg per 100 ml) (Mean of three observations)	2·70	5·05	5·38	7·35	8·08	9·37	9·97	10·32	10·80	13·67	8·267
Difference of mean (Manual — trace reader)	+0·13	+0·05	+0·09	+0·18	+0·12	+0·03	+0·06	+0·11	+0·10	+0·10	+0·099

The trace reader was set up with the 10 mg per 100 ml standard.

The day-to-day reproducibility of the two methods was determined by repeated measurements of a group of thirty peaks over 5 days. For convenience of presentation, only one third of the results are shown in Table III. The standard deviations of both methods range from 0·02 to 0·15 mg per 100 ml, and increase with the distance along the chart. This can be attributed to the interval between standardisation and measurement. In addition, a significant systematic difference ($t > 1{\cdot}0$ per cent. significance level) between the two methods of about 0·1 mg per 100 ml is again observed.

As one of the chief advantages of the trace reader is that of labour saving, an attempt was made to assess this in terms of operator time and measurement error rate. A group of forty peaks was measured by three operators using both methods at intervals throughout a working day; the time required for these measurements, excluding setting-up time, was recorded and the standard deviation of the forty observations calculated. In the manual procedure, following normal laboratory practice, the operator read the peaks while an assistant recorded the observations. The results obtained are shown in Table IV. There are four significant features.

TABLE III

DAY-TO-DAY REPRODUCIBILITY OF MEASUREMENTS OF CALCIUM RECORDINGS BY THE MANUAL METHOD AND THE ELECTRONIC TRACE READER

Peak number		3	6	9	12	15	18	21	24	27	30	Mean
Manual (mg per 100 ml) (Five observations)	Mean	5·55	11·03	11·33	11·25	9·83	9·75	10·93	11·28	11·08	11·38	10·34
	S.D.	0·050	0·043	0·044	0·050	0·044	0·050	0·083	0·083	0·083	0·148	0·068
Trace reader (mg per 100 ml) (Five observations)	Mean	5·57	10·88	11·21	11·13	9·83	9·73	10·76	11·14	11·01	11·29	10·25
	S.D.	0·047	0·046	0·017	0·091	0·047	0·069	0·111	0·104	0·143	0·117	0·079
Difference of means (mg per 100 ml) (Manual − trace reader)		−0·02	+0·15	+0·12	+0·12	+0·00	+0·02	+0·17	+0·14	+0·07	+0·09	0·09

The trace reader was set up with the 10 mg per 100 ml standard. Observations are presented in the same sequence as the peaks of the recorder tracing.

TABLE IV

VARIATION OF MEASUREMENT RATE AND STANDARD DEVIATION OF A GROUP OF FORTY PEAKS WITH TIME THROUGHOUT A WORKING DAY

Time		9.30			11.30			14.00			16.00			Mean
Operator	A	B	C	A	B	C	A	B	C	A	B	C	
Manual — Rate (peaks minute^{-1})	40	15	14·5	40	15·1	17·4	40·6	17·8	16·5	43·5	18·8	21·8	
Manual — S.D. of 40 peaks (mg per 100 ml)	1·45	1·45	1·44	1·47	1·46	1·49	1·50	1·52	1·44	1·45	1·49	1·44	1·468
Trace reader — Rate (peaks minute^{-1})	33·5	29·1	13·1	35·4	28·7	15·0	37·4	33·0	17·8	40·0	34·2	23·3	
Trace reader — S.D. of 40 peaks (mg per 100 ml)	1·40	1·38	1·34	1·33	1·31	1·37	1·34	1·37	1·39	1·33	1·33	1·32	1·351
Difference of S.D. (mg per 100 ml) (Manual − trace reader)	+0·05	+0·07	+0·10	+0·14	+0·15	+0·12	+0·16	+0·15	+0·05	+0·12	+0·16	+0·12	+0·117

S.D. = Standard deviation.

(*i*) The standard deviations for both methods were consistent throughout the day and for different operators, implying a constant precision of operation.

(*ii*) The standard deviation of the trace-reader measurements was systematically 0·05 to 0·16 mg per 100 ml less than that of the manual method, which suggests that the former is the more accurate method. This difference, which is highly significant ($t > 0{\cdot}1$ per cent. significance level), could arise from the calibration graphs used in the two methods (Fig. 3), as suggested earlier to account for the systematic difference between the two methods.

(*iii*) Operator skill in both procedures, as shown by the time required for measurements of the same precision, varies considerably from one person to another. There is, however, in all cases a general improvement with practice throughout the day.

(*iv*) There were no detectable fatigue effects with either method, although the observers found the trace reader less demanding in mental effort.

In order that the labour and time-saving aspects of the trace reader could be determined, the time required for the same operator to measure and write his own results was determined. In the case of operator A a measurement rate of 22 peaks minute^{-1} was attained and for operator B 10 peaks minute^{-1}, compared with 40 peaks minute^{-1} and 18 peaks minute^{-1}, respectively, when assistance was available. Further, there is a significant difference in the setting-up time for each method, *i.e.*, 200 s for the manual method and 100 s for the trace reader.

Conclusion

The trace reader described has been in regular use for 6 months and has required no maintenance during that time. The results presented here show that the reproducibility of reading a peak corresponding to a solution containing about 10·0 mg per 100 ml of calcium by the manual procedure ($\pm 0{\cdot}07$ mg per 100 ml) is better than that obtained with the trace reader ($\pm 0{\cdot}08$ mg per 100 ml). However, because of the nature of the calibration procedures in the two methods, the trace reader appears to give more accurate results, as its standard deviation for a group of experimental peaks (mean concentration 8·8 mg per 100 ml) is 0·1 mg per 100 ml less than the corresponding figure for the manual method.

When using the trace reader, a measurement speed equal to (or greater than) the manual method is obtained with half the labour or, alternatively, the rate for a single operator using the chart reader is twice that of the manual method. The digitised data produced by the trace reader can be recorded directly on punched tape for computer processing, thereby eliminating a transcription stage and reducing the risk of errors.

An average measurement rate of 30 peaks minute^{-1} is easily achieved and is sufficient to cope with the needs of most analytical laboratories. With the trace reader, the 500 peaks produced by the simultaneous analysis of 100 samples for five components could be measured by a single operator in 35 minutes, including the time required to set up the instrument.

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Indicator-tube Method for the Determination of Benzene in Air

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A linear colorimetric method for determining benzene vapours in air is described. The method is based on a colour reaction between the benzene vapours and 0·200-mm silica gel treated with 0·7 g ml^{-1} of a 5 per cent. solution of cerium sulphate in fuming sulphuric acid. The glass tubes are filled with indicator powder to a length of 50 mm. The indicator tubes are calibrated experimentally for benzene concentrations of from 0 to 0·3 mg l^{-1} when 500 ml of air are aspirated, and from 0 to 2·0 mg l^{-1} when 100 ml of air are aspirated. The accuracy of the method is from ±15 per cent. for low concentrations of benzene and ±5 per cent. for high concentrations of benzene. The sensitivity is 0·005 mg l^{-1}. The method is not applicable in the presence of toluene and xylene.

MEANS for the determination of benzene vapours in industrial atmosphere are necessary in the interests of protecting the worker.[1] The most suitable methods are those in which indicator tubes are used.[2,3,4,5,6] The indicator tubes produced in West Germany, U.S.A. and Japan[3,5,6] have a relatively low sensitivity, the concentration of the benzene vapours being determined by the colour intensity of the indicator. Indicator-tube methods for determining benzene based on the measurement of the stain length produced are also known.[7,8] Both types of tube are unsatisfactory because of the interference from all aromatic hydrocarbons and oxides of carbon.

The other methods for the determination of benzene are unsuitable for wide practical application, as bulky apparatus and qualified personnel are required.

We have tried to find a quick, precise and specific method for the monitoring of benzene vapours in industrial environments, and have obtained a new colour reaction between the benzene vapours and silica gel that had been treated with cerium sulphate solution.[9] In the reaction a bluish grey colour is formed.

On the basis of this colour reaction, we have produced an indicator-tube method for determining benzene vapours in air.[10] The development of the method can be divided into the following stages: selection of a suitable support; preparation of the indicator-tube filling; and the filling and calibration of the indicator tubes.

SELECTION OF SUITABLE SUPPORT—

Tests were carried out on quartz sand, porcelain powder and silica gel, but only silica gel gave satisfactory results. Positive results were obtained by using silica gel with a specific surface of 580 m^2 g^{-1} and a maximum distribution of the pores at 50 Å.[9]

Silica gel, 0·200-mm grains (about B.S.I. mesh 72), is treated with nitric acid (1 + 1 v/v), in the ratio of 1 g of silica gel to 3 ml of nitric acid. It is heated on a water-bath for 4 hours, stirring from time to time. The acid is poured off and the silica gel washed thoroughly with distilled water until no nitrate ions are present; it is then dried at 200° to 210° C for 5 hours. If the silica gel is not snow-white in colour the bleaching must be repeated.

PREPARATION OF THE INDICATOR-TUBE FILLING—

Five grams of cerium sulphate, $Ce(SO_4)_2.4H_2O$, are transferred into a 200-ml reaction flask and 100 ml of fuming sulphuric acid introduced. Glass beads are added, the flask closed and its contents stirred vigorously until the cerium sulphate has completely dissolved to form a red - orange solution. The solution should be used immediately, as a fine yellow - orange sediment forms on standing.

To the prepared silica gel add 0·7 ml g^{-1} of 5 per cent. cerium sulphate solution, the silica gel being stirred vigorously between additions of solution so that an even distribution of the reagent on the support is obtained. After the final addition of solution has been made the silica gel is stirred for 1 minute and sealed immediately into glass containers for storage. The orange - yellow powder is stable for about 1 year.

FILLING AND CALIBRATION OF THE INDICATOR TUBES—

The filling is introduced into glass tubes, 100 mm in length and 4·0 mm i.d., to a length of 50 mm. Pads of glass-wool, 1 to 2 mm thick, are inserted in both ends of the tubes. The packing procedure for each tube must be standardised to some reproducible lengths at any particular concentration of benzene vapour.[11] Finally both ends of the indicator tubes are sealed.

TABLE I

RELATIONSHIP BETWEEN THE CONCENTRATION OF THE BENZENE VAPOUR AND STAIN LENGTH

Concentration of benzene vapour, mg l^{-1}	Length of the coloured stain, mm	Aspirated air, ml	Concentration of benzene vapour, mg l^{-1}	Length of the coloured stain, mm	Aspirated air, ml
0·005	2	500	0·13	5	100
0·01	4	500	0·27	10	100
0·02	8	500	0·5	17	100
0·05	14	500	1·0	28	100
0·10	24	500	1·5	38	100
0·20	37	500	2·0	48	100
0·30	50	500			

The calibration of the indicator tubes is carried out for benzene concentrations of from 0 to 0·3 mg l^{-1}, when 500 ml of air are aspirated, and from 0 to 2·0 mg l^{-1}, when 100 ml of air are aspirated through the indicator tubes. A Dräger-type aspirator, which is of Polish production, is used. With one single aspiration, 100 ml of air pass through the indicator tubes, so that for the calibration of the first limit, five aspirations are carried out and only one for the second limit. Several different concentrations are prepared for each limit and five or six determinations are carried out for each concentration. The relationship between the concentration of the benzene vapours and the stain length is shown in Table I. For comparison, control determinations of separate concentrations are carried out by the classical laboratory method[1] as shown in Table II. The accuracy of the method is not affected by air humidity, or by temperature over the range −30° to +30° C.

TABLE II

COMPARISON OF THE DETERMINATION OF BENZENE VAPOURS BY A PHOTOMETRIC AND THE PROPOSED INDICATOR-TUBE METHODS

Nominal concentration of benzene vapours, mg l^{-1}	Concentration of benzene vapour, mg l^{-1}, found by		Error, per cent.
	photometric method	indicator-tube method	
0·05	0·05	0·05	0
0·10	0·10	0·09	− 10
0·20	0·21	0·22	+ 4·5
0·30	0·31	0·34	+12·9
0·70	0·68	0·67	− 1·32
1·50	1·50	1·50	0
2·00	2·03	2·10	+ 3·44

DISCUSSION

The method has been found satisfactory in industrial premises, in which benzene is used. When the results obtained by this method are compared with those obtained by the photometric method it is found that the accuracy for low concentrations is ±15 per cent. and for high concentrations is ±5 per cent. (Table II). The sensitivity of the method is 0·005 mg l^{-1} of benzene (the maximum permissible concentration of benzene vapour in industrial air is 0·01 mg l^{-1}), at which concentration a 2-mm long coloured stain is obtained.

Conclusions

The linear colorimetric method for determining benzene vapour is based on a new colour reaction, and can be applied in the presence of different hydrocarbons and carbon monoxide, but is not applicable in the presence of toluene and xylene vapours. The sensitivity of the method is 0·005 mg l^{-1} and the accuracy is ± 5 per cent. for high concentrations and ± 15 per cent. for low concentrations of benzene vapours. As the method does not require the preparation of special apparatus or qualified workers it is especially suitable for wide practical application.

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The Detection of Prophylactic Drugs in Animal Feeding Stuffs by Thin-layer Chromatography

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A procedure is described for detecting the presence of seventeen prophylactic drugs commonly present in animal feeding stuffs. Only two solvent systems are used for separation purposes. The drugs investigated were: acinitrazole, 2-chloro-4-nitrobenzamide (aklomide), aminonitrothiazole, amprolium, N^4-acetyl-4′-nitrosulphanilanilide (APNPS), buquinolate, decoquinate, dimetridazole, 3,5-dinitrobenzamide (DNBA), ethopabate, furazolidone, clopidol (metichlorpindol), nitrofurazone, pyrimethamine, methyl benzoquate, sulphaquinoxaline and 3,5-dinitro-*o*-toluamide (zoalene).

THE number and complexity of the additives present in modern animal feeding stuffs constitute a problem for the analyst. Although the nature and content of some additives may be declared, it is often necessary to confirm their presence, and to check whether others are present before proceeding with a particular method of quantitative assay. Any procedure that is devised to detect the presence of such additives will be limited in scope to those provided for in the scheme, and consideration must be given to changes in practice as new substances are introduced and older substances pass out of use. The Fertilisers and Feeding Stuffs Regulations 1968[1] require the declaration of the contents of any coccidiostat or blackhead remedy added to a feeding stuff, and the scheme described here is proposed as a preliminary screening procedure as an aid in support of subsequent quantitative analysis. Knapstein[2] has given details of the identification of zoalene, nitrofurazone, furazolidone, amprolium and furnicozone (5-nitrofurfurylideneisonicotinohydrazide) by thin-layer chromatography, and has described methods for the quantitative separation of pairs of these substances when they occur in mixtures. Smith and Thiegs[3] have published colorimetric procedures for the identification of coccidiostats in pre-mixes and finished feeds, and list twelve compounds to which they can be applied. Antkowiak and Spatorico[4] have applied the thin-layer chromatographic technique to the identification of eighteen ingredients and seven mixtures commonly used in medicated feeds, using twelve solvent systems and eight detection agents, but their paper relates to pure drugs and not to isolates from commercial feeds. The present paper extends the basic work of Knapstein and has been developed to cover seventeen prophylactic drugs which are currently in use. Only two solvent systems and four detection agents, together with inspection by ultraviolet light, are used, and the additives, which can be separated and identified, are listed in Table I. The estimated minimum level of commercial usage quoted has been collated from various sources and is given for guidance purposes as to the possible content of an additive in a feeding stuff. The detection procedure may not be sufficiently sensitive for certain drugs when present only in minimal amounts, as, for example, ethopabate at 2 p.p.m., which may occur at this level when present in mixtures with other coccidiostats.

METHOD

REAGENTS—

All chemicals should be of laboratory-reagent grade.

Alumina—Woelm neutral aluminium oxide. Stir 100 g with 200 ml of water for 5 minutes. Allow to settle and decant the supernatant liquor. Repeat twice more with 100-ml portions of water. Collect the slurry on a filter-paper in a Buchner funnel and wash the alumina on the filter with two portions of methanol, and air dry by maintaining suction. This should be freshly prepared each week.

Silica gel G—Available from E. Merck. Immediately before use shake 30 g with 60g of water for 1 to 2 minutes. This amount will be sufficient to coat six plates, 200 × 200 mm.

Acetonitrile.

Chloroform.

Development solvent A—Add 9 volumes of chloroform to 1 volume of methanol and mix.

Development solvent B—Add 1 volume of ethanol to 1 volume of N hydrochloric acid.

Detection agent A—1,2-Diaminoethane.

Detection agent B—Dragendorff's reagent according to Thies, Reuther and Vagujfalvi (as quoted in Stahl[5]) stock solution. Add 2·6 g of bismuth iodide and 7·0 g of sodium iodide to 25 ml of glacial acetic acid and boil for a few minutes. Allow it to cool and stand overnight. Filter off the precipitated sodium acetate on a glass sinter. Add 8 ml of ethyl acetate to 20 ml of the filtrate and store in a dark glass bottle. This solution may not keep longer than 2 to 3 months.

Working solution for spraying—Mix 10 ml of the stock solution with 25 ml of glacial acetic acid and 60 ml of ethyl acetate. The detection sensitivity of this reagent is increased by spraying the plate finally with 0·1 N sulphuric acid (the background becomes grey and the spots darken).

Detection agent C—Ehrlich's reagent. Dissolve 1 g of 4-dimethylaminobenzaldehyde in 30 ml of hydrochloric acid (sp.gr. 1·18) and add 180 ml of butanol.

Detection agent D, diazotisation solutions—

(*a*) Dissolve 10 g of tin(II) chloride in 25 ml of hydrochloric acid (sp.gr. 1·18) and add 10 ml of water.

(*b*) Dissolve 0·1 g of sodium nitrite in 50 ml of 0·1 N hydrochloric acid.

(*c*) Dissolve 0·1 g of *N*-1-naphthylethylenediamine dihydrochloride in 50 ml of water.

Spray with reagent (*a*) and heat plate for 4 minutes at 100° C and allow to cool, then spray with reagent (*b*) and allow to stand for 2 minutes; finally spray with reagent (*c*).

Detection agent E, citric acid - boric acid mixture[6]—Dissolve 0·5 g of citric acid monohydrate and 0·5 g of boric acid in 20 ml of water.

TABLE I

ADDITIVES COVERED BY SCHEME OF DETECTION (GROUPED TOGETHER ACCORDING TO STRUCTURAL SIMILARITY)

	Common name	Estimated minimal commercial usage, p.p.m.	Systematic name
1.	Acinitrazole	150	2-Acetamino-5-nitrothiazole
2.	Aminonitrothiazole	—	2-Amino-5-nitrothiazole
3.	Furazolidone	10	3-(5-Nitrofurfurylideneamino)-2-oxo-oxazolidine
4.	Nitrofurazone	60	5-Nitrofurfuraldehyde semicarbazone
5.	Aklomide	150	2-Chloro-4-nitrobenzamide
6.	DNBA	—	3,5-Dinitrobenzamide
7.	Zoalene	40	3,5-Dinitro-*o*-toluamide
8.	Ethopabate	2	Methyl 4-acetamido-2-ethoxybenzoate
9.	APNPS	200	N^4-Acetyl-4′-nitrosulphanilanilide
10.	Sulphaquinoxaline	20	2-(*p*-Aminobenzenesulphamido)quinoxaline
11.	Buquinolate	100	Ethyl 6,7-di-isobutoxy-4-hydroxyquinoline-3-carboxylate
12.	Decoquinate (deccox)	30	Ethyl 6-decyloxy-7-ethoxy-4-hydroxy-quinoline-3-carboxylate
13.	Methyl benzoquate (statyl)	10	Methyl 7-benzyloxy-6-butyl-1,4-dihydroxy-quinoline 3-carboxylate
14.	Amprolium	30	1-(4-Amino-2-propyl-5-pyrimidylmethyl)-2-picolinium chloride hydrochloride
15.	Dimetridazole	125	1,2-Dimethyl-5-nitroimidazole
16.	Clopidol (meticlorpindol)	125	3,5-Dichloro-2,6-dimethylpyridin-4-ol
17.	Pyrimethamine	10	2,4-Diamino-5-(4-chlorphenyl)-6-ethyl-pyrimidine

APPARATUS—

Chromatographic column—This is about 300 mm long with an i.d. of 10 mm, constricted at one end to an i.d. of 4 mm. Insert a small plug of cotton-wool above the constriction, and

add 5 g of alumina prepared as described under Reagents. Tap the column gently during the addition. The length of the alumina packing will be about 50 mm.

Chromatographic plates—200 × 200 mm, coated to a thickness of 0·25 mm with silica gel G, prepared as described. Activate the plate before use by heating to 100° C for 1 hour.

Chromatographic tanks—Desaga or other similar types, with an internal liner of filter-paper. The tanks are equilibrated before use with 100 ml of the prepared solvent system.

PREPARATION OF SAMPLE—

Grind about 100 g of the sample so that it passes through a sieve with 1 mm square apertures. (A British Standard Test Sieve, Mesh No. 16, is suitable.)

EXTRACTION OF THE DRUGS—

For all drugs listed except amprolium—Weigh 10 g of the prepared feeding stuff and add 40 ml of a mixture of acetonitrile and chloroform, prepared by taking 32 ml of acetonitrile and making up to 40 ml with chloroform. Stir the mixture for 1 hour. Pour the slurry as completely as possible into a 50-ml centrifuge tube and centrifuge at about 2500 r.p.m. for 3 minutes. Decant the supernatant liquid on to the chromatographic column and collect the eluate in a 50-ml beaker at a rate of about 2 drops second^{-1}. This is fraction 1. Then pass 5 ml of chloroform down the column (fraction 2) followed by 40 ml of chloroform (fraction 3) and 10 ml of methanol (fraction 4). Discard fraction 2 but collect fractions 1, 3 and 4 separately. Evaporate fraction 1 to dryness on a steam-bath under a stream of air, avoiding excessive heating (this operation must be carried out in a fume cupboard as acetonitrile is toxic). Re-dissolve the residue in about 2 ml of chloroform - acetonitrile (1 + 1). Evaporate fractions 3 and 4 to a volume of 2 ml under a stream of air. Transfer 20 to 25 μl of each fraction to prepared plates in four groups of three, and a fifth group from fraction 3, so that each group can be treated with a particular spray reagent. Develop the plates in solvent A until the solvent rises by 150 mm. Remove from the tank, allow the plate to air dry and examine under ultraviolet light (254 nm band) and note the response. Spray one group with detection agent A, the other three groups with detection agents B, C and D, and fraction 3 in the fifth group with detection agent E. Interpret the results with the aid of Table II. The R_F values should not be regarded as absolute as they may be influenced by the presence of other extractives from the feed. For this reason, the identity of an isolate should be confirmed by re-chromatographing the suspected material against known standards.

TABLE II

IDENTIFICATION OF ADDITIVES

Additive	Fraction	R_F value	Colour developed with: Reagent A (diaminoethane)	Reagent B (Dragendorff's)	Reagent C (Ehrlich's)	Reagent D (diazotisation solutions)	Ultraviolet fluorescence
Acinitrazole ..	1	0·52	Yellow	—	—	—	Dark spot
Aklomide	1	0·48	—	—	Yellow	Red - purple	Dark spot
Aminonitrothiazole	1	0·32	Red	Red	—	—	Dark spot
APNPS	4	0·40	Yellow	Yellow - brown	Yellow	—	Dark spot
Buquinolate ..	3	0·47	—	Dull orange	—	—	Violet fluor
Deccox	3	0·53	—	Yellow - orange	—	—	Blue fluor
Dimetridazole ..	1	0·61	—	Red	Orange	—	Dark spot
DNBA	1	0·40	Red - purple	—	—	Red - purple	Dark spot
Ethopabate ..	1	0·51	—	—	Dull red	—	Violet fluor
Furazolidone ..	1	0·45	Dark buff	Yellow	—	—	Brown
Meticlorpindol ..	4	0·32	—	Dull red	—	—	Dark spot
Nitrofurazone ..	4	0·25	Red buff	Dull yellow	—	—	Brown
Pyrimethamine ..	4	0·30	—	Orange	—	—	Violet fluor
Statyl	3	0·37	—	Dull orange	—	—	Blue fluor
Sulphaquinoxaline	4	0·39	—	Dull red	Yellow	—	Dark spot
Zoalene	1	0·37	Purple	—	—	Red - purple	Dark spot

— No reaction.

For amprolium—Weigh 10 g of prepared sample and add 40 ml of methanol. Stir for 15 minutes and centrifuge at 2500 r.p.m. for 3 minutes. Decant the supernatant liquid into a beaker and evaporate to 1 to 2 ml on a steam-bath under a stream of air. Put 20 to 25 μl of solution on to a plate and develop in solvent B. Spray the developed chromatogram with detection reagent B. An orange spot indicates the presence of amprolium.

Discussion

The low R_F values obtained with the development solvents used are advantageous as the drug fractions are well separated from the oil and other matter extracted from the feed materials. This is particularly important for samples with a high oil content, such as hen battery mashes. Examination of the characteristics listed in Table II may give the impression at first sight that certain drugs could be confused or missed if present together. However, in practice, such combinations are unlikely to occur in commercial preparations and those combinations which do occur commercially have been satisfactorily resolved. Ethopabate, if present at levels above 15 p.p.m., and pyrimethamine, if present at 10 p.p.m. or above, will be detected, but they are sometimes present in lower concentrations and they may well be missed by this screening procedure in this event. Zoalene and DNBA, if present together, would not be confused, as the shades of colour given with diaminoethane are markedly different when compared with one another. Separate identification of the hydroxyquinoline derivatives, buquinolate, deccox and statyl, can be achieved in the unlikely event of the presence of two or three being suspected. After viewing by ultraviolet light, the plate is sprayed with detection agent E, the citric acid - boric acid mixture, when the fluorescence of buquinolate and deccox changes to pale blue and statyl changes to violet. This colour change facilitates differentiation and identification of these related compounds. Aklomide and APNPS can be detected by the use of detection agent A, diaminoethane and detection agent D, the diazotisation solutions. Although the main bulk of each appears in the fraction described, some by-products of APNPS sometimes appear in the aklomide fraction and could be mistaken for it. Such by-products also react with detection agent C, Ehrlich's reagent, to give a similar colour to that given by aklomide and in such instances it is necessary to check whether there is clear indication of APNPS in fraction 4. Metichlorpindol gives a poor colouration if present in minimal amounts and caution should be exercised in interpretation. If nitrofurazone is present, furazolidone may be suspected as these substances are frequently used together, yet furazolidone may not show up strongly. Its presence should be confirmed by increasing the spotting fraction to 50 μl, but it should be noted that recourse to such procedure is impracticable for ethopabate or pyrimethamine when the excess of feed extractive masks any attempt at diagnosis. Most of these prophylactic drugs will react with Dragendorff's reagent, but only if they are present in large amounts. However, this reagent should only be relied on to confirm the presence of amprolium, dimetridazole and metichlorpindol.

The following feeding stuffs, taken as a fairly representative selection, were used as carriers for the drugs examined by this procedure: turkey feed, hen battery mash, baby chick mash, layer's mash and high energy layer's mash. Occasionally, chromatograms of feeding stuffs examined in ultraviolet light yield uncharacterised fluorescent spots. These mainly occur in fraction 4 and are unlikely to be confused with drugs isolated in this fraction, especially as they do not give a colour with Dragendorff's reagent.

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Analytical Methods Committee

REPORT PREPARED BY THE PROPHYLACTICS IN ANIMAL FEEDS SUB-COMMITTEE

The Determination of Dimetridazole in Animal Feeds

THE Analytical Methods Committee has received the following Report from its Prophylactics in Animal Feeds Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Prophylactics in Animal Feeds Sub-Committee responsible for the preparation of this Report was: Mr. S. G. E. Stevens (Chairman), Mr. R. J. Anderson, Mr. P. J. Cooper (resigned May, 1967), Mr. A. G. Croft (appointed September, 1967), Mr. G. Drewery, Mr. A. W. Hartley (resigned September, 1967), Mr. R. S. Hatfull, Mr. S. P. Hayes, Mr. D. H. Mitchell, Mr. J. A. Stubbles, Mr. C. B. Stuffins, Mr. D. C. Thomas, Mr. R. E. Weston, Dr. D. R. Williams and Mr. D. R. Wraige, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

Dimetridazole (1,2-dimethyl-5-nitroimidazole) is a drug used for the prevention and treatment of blackhead in turkeys and chickens. It is used mainly as an additive in feeding stuffs at a level of about 125 p.p.m. The drug contains a nitro group that can be reduced to its corresponding free amine and it can, therefore, be determined spectrophotometrically after diazotisation and coupling. Polarography has also been shown to be a rapid and effective method for determining dimetridazole.

EXPERIMENTAL

When the Sub-Committee began its investigation of methods for determining dimetridazole in animal feeds it was decided first to try a polarographic method based on that proposed by Kane.[1] Some of the members of the Sub-Committee found this method to be reasonably satisfactory, but others found that high recoveries were frequently obtained, and co-extraction from the feed of other polarographically reducible species was suspected. Further investigation of polarographic procedures was not carried out at this stage as a diazo-colorimetric method and a thin-layer chromatographic method were tentatively proposed by one of the collaborating laboratories for consideration. The diazo method was based on spectrophotometric measurement after reaction with the Bratton - Marshall[2] reagents and the thin-layer chromatographic method involved visual comparison in ultraviolet light of standard and sample spots that had been sprayed with fluorescein and exposed to bromine vapour. Neither of these methods was found to be particularly satisfactory, although again some members found them to work well. The main difficulty encountered with the diazo method was lack of reproducibility of the calibration graph. With the thin-layer chromatographic method, considerable difficulty was encountered in seeing the spot and in distinguishing between the various levels of standard applied to the plate. Various modifications of this latter method were tried, including removal of the spots from the plate followed by spectrophotometric measurement, but no particular improvement was achieved.

An alternative spectrophotometric method was proposed by one laboratory in which the dimetridazole was extracted from the feed with chloroform, and separated from interfering materials by column chromatography and partition between chloroform and dilute hydrochloric acid. The optical density of the extract was then measured in both acidic and alkaline solution at 315 nm, the difference in readings being referred to a standard solution treated in the same manner. In a collaborative investigation of this method on a feed medicated at the level of 140 p.p.m., six laboratories reported mean recoveries in the range 90 to 104 per cent., but two other laboratories reported low results—from one a mean recovery of 76 per cent. and from the other 66 per cent. Further work by these two laboratories, including an investigation of a modified preliminary extraction procedure, achieved improved recoveries in only one of them.

A preliminary investigation was carried out on a spectrophotometric method proposed by Daftsios.[3] The members did not, however, favour this method, particularly as the solution obtained could not be made sufficiently optically clear for the final measurement. The possibility of applying gas - liquid chromatography to the determination of dimetridazole in animal feeds was also considered by the Sub-Committee. Investigational work in three laboratories indicated that the technique was promising and that good recoveries of the drug could be obtained. It was, however, realised that a considerable amount of development work would be needed before a standardised method could be recommended. At this stage the Sub-Committee decided not to pursue the gas-chromatographic work further, but instead to renew its interest in polarographic methods.

Since the Sub Committee began its work the A.O.A.C. has recommended a polarographic method for dimetridazole,[4] and a manufacturer of the drug also recommended a polarographic method, based on the work of Kane[1] for its determination in turkey feed. Individual work in some laboratories had suggested that the A.O.A.C. method was more tedious than the method recommended by the manufacturer and, in consequence, a collaborative investigation of the latter method was arranged. Three blank feeds were obtained from different manufacturers and medication was carried out within each laboratory taking part. The results of these tests are shown in Table I.

TABLE I

RECOVERY OF DIMETRIDAZOLE FROM ANIMAL FEEDS BY A POLAROGRAPHIC METHOD

Feed	Laboratory	Dimetridazole added, p.p.m.	Dimetridazole found, p.p.m.	Recovery, per cent.
I	A	125	126	101
		125	117	94
	B	130	272	209
		124	288	232
	C	117	194	166
		123	263	215
	D	175	138	79
	E	125	116	93
		125	136	109
		125	125	100
II	B	129	152	118
		120	133	111
	C	119	118	99
	D	105	113	108
		120	132	110
	E	125	148	119
III	A	125	124	99
		125	128	102
	B	131	132	101
		132	136	103
	C	121	122	101
		121	117	96
		119	121	101
	D	175	179	102
		115	117	102
	E	125	140	112

From these results it appeared that the constituents of the feeds had a considerable effect on the polarographic determination of dimetridazole. On enquiry, differences between the feeds were discovered in that feed I was a normal commercial production feed, feed II contained no fat and considerably less added minerals than feed I, and feed III contained no added minerals or vitamins.

In the first instance it was thought that the high results may have been due to the presence of iron or phosphate. A second sample of blank feed containing no added minerals was obtained from the manufacturer of feed I and recoveries of dimetridazole from this with and without the addition of 1 and 2 per cent. of dicalcium phosphate were satisfactory. Similarly, the presence of up to 500 p.p.m. of iron was found to have no apparent effect on the recovery of dimetridazole.

One of the laboratories that had earlier obtained high results for the recovery of the drug from feed I decided to carry out a rigorous investigation of the effects of the presence of other metal ions on the polarographic determination of dimetridazole. In this work it was shown that the presence of copper had a marked effect on the peak height for dimetridazole and on the background to the peak. It was also shown that the presence in the feed of up to 400 p.p.m. of iron, nickel and zinc had no effect on the dimetridazole wave. This work was reported by Cooper and Hoodless,[5] who overcame the interference from copper by complexing it with potassium cyanide after the addition of sodium borate to the acidic extract from the feed. The Sub-Committee has subsequently shown that the presence of up to 650 p.p.m. of manganese in the feed has no effect on the dimetridazole wave.

Before undertaking a full collaborative test of Cooper and Hoodless's method, four laboratories undertook an investigation of the method on four feed samples, two from each of two different manufacturers. Medication was carried out in each laboratory and the results obtained are shown in Table II. Laboratory C also determined the recovery of dimetridazole from two of the feeds omitting the potassium cyanide treatment; the results obtained were, for feed IV 159 per cent. and for feed V 217 per cent.

TABLE II

DETERMINATION OF DIMETRIDAZOLE IN FOUR FEEDS BY THE MODIFIED POLAROGRAPHIC METHOD PROPOSED BY COOPER AND HOODLESS

Feed*	Laboratory	Dimetridazole added, p.p.m.	Dimetridazole found, p.p.m.	Recovery, per cent.
IV	A	125	97	78
	C	118	115	97
		116	114	98
	E	112	108	97
	F	125	156	125
		125	167	134
V	A	125	117	94
	C	127	121	95
		128	122	95
	E	105	100	95
	F	125	156	125
		125	130	104
VI	A	125	111	89
	C	131	133	102
		133	139	103
	E	233	234	100
		98	94	96
	F	127	137	112
		127	167	134
VII	A	125	124	99
	C	118	118	100
		128	121	94
	E	105	108	103
		116	110	95
	F	126	127	101

**Added mineral contents of feeds—*

Feed IV—Blank meal *plus*

Copper	100 p.p.m.
Manganese	100 p.p.m.
Zinc	90 p.p.m.
Iron	200 p.p.m.

Feed V— Blank meal IV *plus*

Copper	150 p.p.m.
Manganese	100 p.p.m.
Zinc	90 p.p.m.
Iron	200 p.p.m.

Feed VI—Blank meal only

Feed VII—Blank meal VI *plus*

Calcium	2 per cent.
Phosphorus	1·2 per cent.
Magnesium	0·1 per cent.
Iron	500 p.p.m.
Manganese	300 p.p.m.
Copper	250 p.p.m.
Zinc	125 p.p.m.

The results from Laboratory F were not concordant with those returned by the other three laboratories and in consequence this laboratory consulted with one of the other laboratories in an attempt to solve its difficulties. Further work by Laboratory F then produced satisfactory recoveries of dimetridazole, although the reason for the erratic recoveries obtained earlier was not discovered.

A statistically designed collaborative test was then carried out in six laboratories. Two feed samples were used, one containing only naturally occurring trace elements and the other being the same blank feed containing added minerals. Medication of these blank feeds was again carried out in each of the laboratories taking part and the results, together with statistical treatments of them, are given in Tables IIIA, IIIB, IV, V and VI.

TABLE IIIA

RESULTS OF THE STATISTICALLY DESIGNED COLLABORATIVE TEST OF THE METHOD PROPOSED BY COOPER AND HOODLESS

Medication levels, approximately 180 p.p.m. of dimetridazole; weight of sample, 40 g

		Feed sample VIII*					Feed sample IX*				
		Drug added		Drug found		Re-covery,	Drug added		Drug found		Re-covery,
Laboratory	Polarograph	mg	p.p.m.	mg	p.p.m.	per cent.	mg	p.p.m.	mg	p.p.m.	per cent.
A	Davis C.R.	7·60	190·0	7·76	194·0	102·1	7·12	178·0	7·00	175·0	98·3
		7·32	183·0	7·44	186·0	101·6	7·00	175·0	6·88	172·0	98·3
		7·56	189·0	7·44	186·0	98·4	7·12	178·0	6·88	172·0	96·6
B	Davis C.R.	7·15	178·8	6·80	170·0	95·1	7·41	185·3	6·80	170·0	91·7
		7·42	185·5	7·60	190·0	102·4	7·25	181·3	7·70	192·5	106·2
		7·12	178·0	7·65	191·3	107·5	7·23	180·8	6·65	166·3	92·0
C	Davis C.R.	7·18	179·5	7·05	176·3	98·2	7·24	181·0	6·94	173·5	95·9
		7·10	177·5	6·88	172·0	96·9	7·04	176·0	6·66	166·5	94·6
		7·47	186·8	7·05	176·3	94·4	7·50	187·5	6·38	159·5	85·1
D	Southern	7·14	178·5	7·71	192·8	108·0	7·23	180·8	7·68	192·0	106·2
	Manual	7·22	180·5	7·06	176·5	97·8	7·21	180·3	7·44	186·0	103·2
		7·23	180·8	8·44	211·0	116·7	7·24	181·0	8·38	209·5	115·7
E	Southern	7·17	179·3	7·79	194·8	108·6	7·20	180·0	6·98	174·5	96·9
	Manual	7·10	177·5	6·45	161·3	90·9	7·29	182·3	7·66	191·5	105·0
		7·25	181·3	7·45	186·3	102·8	7·16	179·0	7·48	187·0	104·5
F	Southern	7·20	180·0	7·25	181·3	100·7	7·20	180·0	7·34	183·5	101·9
	Manual	7·20	180·0	6·88	172·0	95·6	7·20	180·0	7·46	186·5	103·6
		7·20	180·0	7·08	177·0	98·3	7·20	180·0	7·30	182·5	101·4

**Mineral contents of feeds*—*

Feed sample VIII contained natural minerals only, *viz.*, copper 8 p.p.m., iron 100 p.p.m., manganese 28 p.p.m. and zinc 43 p.p.m.

Feed sample IX contained added minerals giving concentrations of copper 213 p.p.m., iron 1495 p.p.m., manganese 716 p.p.m. and zinc 851 p.p.m.

TABLE IIIB

ANALYTICAL RESULTS OF COLLABORATIVE TEST EXPRESSED AS A PERCENTAGE RECOVERY SHOWING TOTALS AND MEAN VALUES

	Treatment 1 (Sample VIII)		Treatment 2 (Sample IX)			Over-all
Laboratory	Recovery, per cent.	Mean, per cent.	Recovery, per cent.	Mean, per cent.	Sum	mean, per cent.
A	102·1		98·3			
	101·6		98·3			
	98·4		96·6			
	302·1		293·2		595·3	
		100·7		97·7		99·2
B	95·1		91·7			
	102·4		106·2			
	107·5		92·0			
	305·0		289·9		594·9	
		101·7		96·6		99·2

TABLE IIIB—*continued*

C	98·2		95·9			
	96·9		94·6			
	94·4		85·1			
	289·5		275·6		565·1	
		96·5		91·9		94·2
D	108·0		106·2			
	97·8		103·2			
	116·7		115·7			
	322·5		325·1		647·6	
		107·5		108·4		107·9
E	108·6		96·9			
	90·9		105·0			
	102·8		104·5			
	302·3		306·4		608·7	
		100·8		102·1		101·5
F	100·7		101·9			
	95·6		103·6			
	98·3		101·4			
	294·6		306·9		601·5	
		98·2		102·3		100·3
Totals	1816·0		1797·1		3613·1	
Over-all mean		100·9		99·8		
Over-all total			3613·1			

TABLE IV

ANALYSIS OF VARIANCE DERIVED FROM RESULTS SHOWN IN TABLE IIIB

Source of variation	Sums of squares	Degrees of freedom	Mean square	*F* Ratio
Total variance	1495·262	35		
Due to laboratories	596·867	5	119·373	3·65*
Due to samples (treatments)	9·922	1	9·922	0·30
Due to interactions of laboratories × samples	102·612	5	20·522	0·63
Residual variance	785·861	24	32·744	

	Critical values of *F*	
Level of significance	P = 0·05	P = 0·01
For degrees of freedom 5 and 24	2·62	3·90
For degrees of freedom 1 and 24	4·26	7·82

*As the calculated value of *F* for the variance between laboratories at 3·65 exceeds the critical value at 2·62 for a significance level of 0·05, the variance between laboratories is slightly greater than the residual or random variance. This is not thought to be excessive, and the test procedure can be considered sufficiently precise for practical use. The chance of differences such as these arising is once in twenty times.

TABLE V

COMPARISON OF MEAN VALUES

Mean	Minimum difference for a significance at P = 0·05	Inference
Comparison of treatments within laboratories	9·62	None significant
Comparison of laboratory means over both treatments	10·21	Lab. C and Lab. D significantly different*
Comparison of laboratory means within one treatment	14·44	None significant

* The significant difference between laboratory C and laboratory D confirms the significant laboratory effect shown by the variance ratio in Table IV.

TABLE VI

OVER-ALL PERFORMANCE OF METHOD

No. of samples tested	36
Mean recovery, per cent.	100·4
Range { Min.	85·1
Range { Max.	116·7
Coefficient of variation	6·5
95 per cent. confidence limits for a single test in terms of percentage recovery	87·4 to 113·4

From a consideration of the results in Table IIIA and of the statistical evaluation of these results the Sub-Committee recommends that the modification by Cooper and Hoodless of Kane's polarographic method be used for the determination of dimetridazole in animal feeding stuffs. Full details of the procedure are given in Appendix I.

RECOVERY OF DIMETRIDAZOLE FROM PELLETED FEEDS—

After the Sub-Committee had completed its work an investigation of the recovery of dimetridazole from pelleted feeds was carried out by the Compound Animal Feedingstuffs Manufacturers National Association Ltd. (CAFMNA). The results obtained in this work are given in Appendix II.

Appendix I

RECOMMENDED METHOD FOR THE DETERMINATION OF DIMETRIDAZOLE IN ANIMAL FEEDING STUFFS

PRINCIPLE OF THE METHOD—

The method is that recommended by Cooper and Hoodless.[5] The sample is extracted with 0·65 per cent. hydrochloric acid and the acid extract is treated with sodium tetraborate and then with potassium cyanide to complex any copper present. The solution is polarographed and the peak height at —0·35 V is measured. The dimetridazole content is calculated from the difference between this peak height and that for a similarly treated solution containing a known amount of added dimetridazole.

APPLICABILITY—

The method is suitable for determining the usual concentrations of dimetridazole added to feeds. There is not likely to be any interference from copper, iron, nickel, zinc and manganese present in the feed up to the levels indicated in this Report.

REAGENTS—

Potassium cyanide solution, 5 per cent. w/v.

Sodium tetraborate.

Dimetridazole standard solution—Dissolve 0·1 g of pure dimetridazole in 200 ml of water containing 5 ml of dilute hydrochloric acid.

Hydrochloric acid, 0·65 per cent. w/v—Dilute 17·6 ml of concentrated hydrochloric acid (sp.gr. 1·18) to 1 litre with water.

PROCEDURE—

To 40 g of feeding stuff in a 500-ml flask add 320 ml of 0·65 per cent. w/v hydrochloric acid, and stir the mixture for 2 hours. Allow to settle for 1 hour. Transfer by pipette 1 ml of water and 1 ml of dimetridazole standard solution, separately, into two 25-ml flasks. Make both up to volume with separated feed extract solution. Transfer the solutions to two dry centrifuge tubes, and shake them with 3 g of sodium tetraborate for 1 minute. Add 1 ml of 5 per cent. w/v potassium cyanide solution, and shake the tubes to mix. Spin them in a centrifuge at 3000 to 4000 r.p.m. for about 3 minutes.

Transfer sufficient solution from the two tubes to dry polarographic cells and de-oxygenate the solutions with oxygen-free nitrogen. Record the polarograms of the solutions from —0·05 to —0·55 V, or over a suitable potential range to measure the peak heights at —0·35 V. Calculate the dimetridazole content of the sample solution from the difference between the peak heights of the two solutions.

Appendix II

Recovery of dimetridazole from pelleted feeds

A collaborative investigation of the recovery of dimetridazole from pelleted feeds was carried out under the auspices of the Compound Animal Feedingstuffs Manufacturers National Association Ltd. A report of this work is given below.

Objective—

To test the suitability of the method recommended in Appendix I for application to both pelleted and unpelleted feeds.

Experimental—

A 5-ton batch of turkey feed was made on a plant that had been cleaned out by the manufacture of a similar batch immediately beforehand. A sampling scheme was designed so that ten spot samples of meal were taken both before and after pelleting. These two series of samples were each separately bulked and mixed to provide average samples of the feed before and after pelleting.

Analytical method—

The method used was that given in Appendix I. Bulk samples of the material before and after pelleting were sent to three laboratories. Each laboratory prepared three sub-samples of both batches and analysed each sub-sample singly.

Results—

Table VII

General analysis of samples

	Oil, per cent.	Protein, per cent.	Fibre, per cent.	Moisture, per cent.	Ash, per cent.	Salt, per cent.
Before pelleting ..	5·6	24·3	2·6	12·2	5·6	0·6
After pelleting	5·6	24·4	2·9	10·9	5·8	0·6

Table VIII

Recovery of dimetridazole before and after pelleting

All results corrected to 11·6 per cent. mean moisture level
Theoretical formulation level = 150 p.p.m. of dimetridazole

	Dimetridazole found	
Laboratory	Before pelleting, p.p.m.	After pelleting, p.p.m.
I	155, 154, 152	151, 152, 149
II	156, 158, 173	163, 156, 156
III	163, 151, 142	145, 142, 134
Mean	156	150
Standard deviation	8·54	8·60

Conclusions—

The mean levels of dimetridazole in the meal before and after pelleting are not significantly different. The standard deviations before and after pelleting are not significantly different.

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Recommended Methods for the Evaluation of Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS FOR THE EVALUATION OF DRUGS

The Assay of Phenothiazine

In January, 1964, the Joint Committee of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry appointed a working panel to examine methods for the assay of phenothiazine. The constitution of the Panel was: Mr. W. H. Stephenson (Chairman), Mr. J. K. Bailey, Dr. P. Casapieri (resigned December, 1966), Mr. A. Holbrook, Mr. D. A. Walker and Mr. R. J. Anderson (appointed December, 1966), with Mr. P. W. Shallis as Secretary. The terms of reference of the Panel were "To recommend a satisfactory method for determining phenothiazine in commercial phenothiazine and to extend this method to cover most commercial phenothiazine dispersible powders." At a later date the Panel extended its terms of reference to include phenothiazine liquid formulations but excluded compound phenothiazine preparations.

Introduction

Phenothiazine is used extensively in veterinary medicine as an anthelmintic, being effective against a wide range of gastro-intestinal nematodes. The precise mode of action of the drug is unknown, but particle size and purity greatly influence its efficacy in ruminants. It is generally prepared by heating diphenylamine with an excess of sulphur or sulphur chloride in the presence of a trace of iodine as catalyst. Diphenylamine has been identified as an impurity (about 0·5 per cent.) in some commercial phenothiazine. Oxidation products, phenothiazine sulphoxide and sulphone may be present and a small amount of carbazole may sometimes be associated with the drug.

The major impurity is thought to be a mixture of polymeric forms of phenothiazine formed by further reaction with sulphur, but the true nature of these impurities is not known with certainty. They can be present in amounts up to 20 per cent. in crude phenothiazine samples.

Several colorimetric methods of assay have been proposed and these have been examined by Gunew,[1] who commented on their disadvantages. He proposed a chromatographic method that was somewhat complex and lengthy and required a special absorbent, which was difficult to obtain. Brierley and Langbridge[2] proposed an absorption chromatographic technique in which phenothiazine was separated from impurities by absorption on to an acidic aluminium oxide column followed by elution with a mixture of diethyl ether and light petroleum. The phenothiazine was determined by measurement of its extinction at 254·5 nm.

A partition chromatographic method was described by Holbrook, Barlow and Bailey[3] using an acetonitrile - hexane system supported on Celite. The phenothiazine was determined by measurement of the extinction of the eluate at 253 nm.

The method adopted by the British Veterinary Codex 1953 was a determination of the total nitrogen by means of a Kjeldahl-type digestion and distillation. This was non-specific and did not adequately differentiate between samples with varying phenothiazine contents, as much as the "polymerised impurity" was calculated as phenothiazine.

The objective of the Panel was to produce a method of assay that would

(1) be suitable for inclusion in a future edition of the British Veterinary Codex,

(2) give a satisfactory indication of the true phenothiazine content of commercial grades of phenothiazine,

(3) be able to be used directly or with a minimum of modification to assay the phenothiazine content of commercially available formulations of phenothiazine.

Because of the undetermined nature of most of the impurities content of technical material, the Panel favoured a chromatographic method of assay as being the most likely

to differentiate between phenothiazine and these associated impurities, but any technique that could be of assistance and which was available to the Panel would be examined.

Based on the experience of Panel members the method of Gunew was rejected as being extremely tedious and unsuitable for an industrial laboratory procedure. It was decided, therefore, to concentrate attention on the absorption method of Brierley and Langbridge and the partition method of Holbrook, Barlow and Bailey, but to investigate the applicability of a method utilising the infrared absorption at 922 cm^{-1} and 1260 cm^{-1}, which had been suggested by a Panel member as a possible assay technique.

At a fairly early stage in the investigations it was learned that the Editor of the British Veterinary Codex proposed to include the partition chromatographic method of Holbrook, Barlow and Bailey as an assay procedure for phenothiazine when the 1965 edition was published. Certain of the modifications proposed by the Panel were incorporated before publication.

Experimental

Two reference samples of phenothiazine were prepared—

(*a*) by distillation followed by recrystallisation,

(*b*) by repeated vacuum sublimation.

These two samples differed in colour and in their extinction coefficients at 253 nm [Sample (*a*) $E^{1\%}_{1cm}$ 253 nm 2210, Sample (*b*) $E^{1\%}_{1cm}$ 253 nm 2350]. The latter was adopted as the reference preparation.

As the preliminary results by the infrared procedure did not seem to be sensitive to these differences its application was deferred to a later date, attention being given to the evaluation of the two chromatographic methods. It soon became clear that it was necessary to standardise both procedures more precisely, and that purification of reagents and modifications of technique would be required as, although there was good agreement generally between intra-laboratory results, there was some degree of deviation in the inter-laboratory series.

Basic impurities in the acetonitrile gave negative optical density values in the partition method in the hands of some members of the Panel and it had also proved necessary to purify the hexane on occasion. Similarly, the optical density readings in the absorption method had been affected by impurities in the light petroleum. "Clean-up" techniques were evolved for these reagents. It was sometimes difficult to be certain of the exact value for the base-line correction in the partition chromatographic method and this was thought to be due to the slightly irregular elution associated with the evaporation of the sample solution to dryness followed by dissolution of the residue in eluting solvent and quantitative transference to the chromatographic column. The technique was altered so as to place the sample as a solution on to the column with a resulting improvement in the base-line characteristics.

At this stage the Panel felt there was little to choose between the two methods. The absorption technique was easier in that it did not involve the collection of a series of eluate fractions with the associated difficulties of base-line correction, but it had been shown that carbazole was not separated from phenothiazine, and in the examination of samples of unknown origin absence of carbazole could not be assumed. The precision of both methods was not significantly different. The Panel accordingly recommended the adoption of the modified partition chromatographic method of Holbrook, Barlow and Bailey for the purposes of assay of commercial phenothiazine in the British Veterinary Codex (see Appendix I).

During the course of the Panel's work on these two methods, other techniques were suggested and work on some of them was carried out. It had become evident that the accuracy and precision to be expected of methods suitable for the assay of phenothiazine could not be compared with those for materials of higher purity and stability. Hence it was felt that the method utilising the infrared absorbance was worth re-examination, as was a gas - liquid chromatographic technique that had been developed in the laboratory of a Panel member. It was also agreed to investigate the palladium chloride colorimetric method of Ratcliffe and Stevens.[4] This method was devised for the assay of phenothiazine-derived drugs and was stated to be unaffected by the presence of oxidation products.

This method was found to give results in good agreement with those obtained with the modified partition chromatographic method. Some high blank values were encountered and when the reagent was used as a revealing agent in the examination of samples of phenothiazine by thin-layer chromatography, it was shown to produce colours with other associated materials as well as with phenothiazine.

Phenothiazine is readily soluble in carbon disulphide. Its infrared absorbance spectrum over the range 1400 to 650 cm^{-1} exhibits several absorption bands of which those at 922, 1260 and 1300 cm^{-1} might be used as a basis for an assay procedure. Diphenylamine has a strong band at 1250 cm^{-1} and carbazole absorbs at 1240 cm^{-1}. Samples were assayed by the method used earlier (see Appendix II). The spread of results was marginally wider than those obtained by the chromatographic method, those at 1260 cm^{-1} tending to be lower than those calculated from the absorbance at 922 cm^{-1}. Results calculated from the absorbance at 1300 cm^{-1} were lower than either. Cell contamination proved to be an annoying problem and anomalous results were obtained with moderately impure samples. There was evidence of instrumental variation and it was agreed that as a considerable programme of work would be called for to evaluate interferences, and as a gas - liquid chromatographic procedure was showing promise, further investigation of this technique would not be pursued.

Members of the Panel had earlier given thought to the possibility of using gas - liquid chromatography as an assay technique for phenothiazine, and the great improvements in the commercially available apparatus that had taken place during the period of the Panel's work led members to explore its potentialities with encouraging results. A standard method was circulated and all available samples were examined. The preliminary results compared well with those obtained by the partition chromatographic method, but the spread of the results between laboratories was appreciably reduced. The time of analysis was shortened and the ability to carry out a number of assays on a small sample is a favourable feature of the technique.

In the original method di-isopropyl ether was used as a solvent, but because of risk of peroxide formation and associated explosion hazard this was changed to methylene chloride, in which phenothiazine is adequately soluble. It was found that pyrolysis products from this solvent gave rise to carbonisation and some slight corrosion of the detector, although the results were, in all cases, satisfactory. It was thought, however, that an alternative solvent should be sought. As phenothiazine is freely soluble in acetone and the peaks are well separated on a chromatogram it was decided to adopt this solvent with directions that all dilutions should be carried out in actinic glassware and that the chromatogram should be run as soon as possible after the final dilution had been made.

A modified method (see Appendix III) was devised and this gave encouraging results when applied to dispersible powder and dispersible liquid formulations.

Results

At an early stage of the Panel's work a collaborative comparison of the absorption chromatographic[2] and partition chromatographic[3] methods was carried out on one prepared sample theoretically containing 75·5 per cent. of phenothiazine and two samples of technical phenothiazine; the results are shown in Table I.

Table I

Comparison of results by absorption and partition chromatographic methods

Sample	Laboratory	Phenothiazine found, per cent., by Absorption method	Partition method
Prepared (theory 75·5 per cent. of phenothiazine) ..	A	74·4, 74·9, 74·2, 74·4, 75·0	72·6, 73·0
	B	75·9, 76·3	67·0, 68·5
	C	82·9, 81·7	74·6, 74·4
	D	84·7, 80·4	66·4
Technical	A	84·7, 84·5, 85·4	88·9, 91·8
	B	87·1, 87·9	92·8, 90·5
	C	93·6, 93·2	91·6, 91·6, 92·2
	D	92·3, 91·6	85·5, 82·5
Technical	A	92·1, 93·1	90·1, 86·9, 90·3, 89·2
	B	89·4, 90·1	92·5, 91·9
	C	91·8, 92·3	92·6, 92·1

TABLE II

COMPARISON OF RESULTS BY THE METHOD GIVEN IN APPENDIX I

Sample	Laboratory	Phenothiazine found, per cent.
Prepared (theory 75·5 per cent. of phenothiazine) ..	A	69·5, 67·2, 71·2, 70·3, 69·5, 69·5
	B	69·0, 70·0
	C	73·5, 74·1
	D	74·1, 73·4
Technical	A	91·5, 92·1, 88·7, 89·8
	B	91·2, 90·2
	C	92·9, 92·8
	D	92·4, 91·1

TABLE III

EXAMINATION OF SAMPLES OF TECHNICAL PHENOTHIAZINE FROM DIFFERENT ORIGINS BY THE INFRARED METHOD DESCRIBED IN APPENDIX II

		Phenothiazine found, per cent., by—			
Sample origin	Laboratory	Infrared at 922 cm^{-1}	Infrared at 1260 cm^{-1}	Partition chromato-graphy	Ratcliffe and Stevens' method
Unknown ..	A	95·8, 97·2	92·8, 93·6		92·7, 93·1, 91·4, 91·8
	B	96·6, 97·2	97·0, 97·0		
	C	99·1, 94·0	94·2, 94·2	92	
	D	93·7	92·5, 93·3, 93·2, 93·8		
British	A	95·9, 95·6	93·1, 91·6		90·8, 91·0, 89·5, 89·6
	B	93·2, 93·8	94·0, 93·8		
	C	94·9, 95·2	94·2, 95·2	91	
	D	93·0	91·3, 93·5, 94·0, 92·1, 93·4, 94·0		
Indian	A	87·6, 86·6, 90·8	87·6, 85·8, 85·6		83·4, 84·4, 82·2, 83·2
	B	90·5, 90·5	89·8, 89·4		
	C	95·0, 96·1, 81·7	93·4, 87·3, 81·0	78·5	
	D	88·7	90·9, 87·1, 88·7, 92·4, 88·0, 89·6		
Australian ..	A	98·9, 98·9	95·1, 95·1		96·7, 97·5, 97·3, 98·0, 96·4, 96·9
	B	95·6, 95·4	95·0, 95·6		
	C	102, 99, 103	99·5, 96·6, 103	96	
	D	95·5	97·0, 98·5, 97·7, 98·5		97·5, 97·3, 97·3
South American ..	A	93·4, 93·0, 94·0	92·3, 91·3, 93·5		89·2, 89·4, 90·1, 90·3
	B	93·0, 94·0	92·8, 93·2		
	C	96·0	95·6	85	
	D	92·8	90·4, 89·8, 92·5, 92·3		85·5, 86·9, 86·6
South African ..	A	76·7, 88·5, 85·2, 89·3, 89·0, 88·2	70·5, 87·5, 80·2, 82·8, 83·1, 83·5, 84·2		75·7, 75·8, 76·5, 76·6, 76·3, 76·6, 76·0, 76·2
	B	87·9, 87·9	85·8, 85·4	88	
	C	89·7	87·5	81	
	D	85·4	82·5, 82·5, 83·7, 82·7, 83·1, 84·2, 84·5, 85·2, 84·2, 84·5		76·0, 76·3, 74·8

A further series of results was obtained on two of these samples by the partition method after some modifications had been introduced. The results are shown in Table II.

Various samples of technical phenothiazine from different origins were obtained and were examined by the infrared method described in Appendix II. The results, calculated from measurements at 922 and 1260 cm^{-1}, are shown in Table III, together with some results by the partition chromatographic method and the Ratcliffe and Stevens method.[4]

Finally, collaborative work on these samples together with some samples of phenothiazine dispersible powders and some of liquid preparations was carried out by the gas - liquid chromatographic method described in Appendix III. Three series of tests were carried out, one with di-isopropyl ether as solvent, one with methylene chloride as solvent and finally with acetone as solvent; the results are shown in Table IV.

TABLE IV

RESULTS OBTAINED WITH DIFFERENT SOLVENTS BY THE METHOD RECOMMENDED IN APPENDIX III

All results are the mean of two or more determinations

Sample	Origin	Lab.	Phenothiazine found, per cent. w/w, with di-isopropyl ether as solvent	methylene chloride as solvent	acetone as solvent
Technical	Unknown	A	91·1	93·3	93·0
		B	91·6	91·1	92·5
		C	92*	92	91·4
		D	88·5	91	
Technical	British	A	88·9	92·2	91·5
		B	92·9	88·6	90·9
		C	92*	91	90·7
		D	87·7	90·7	
Technical	Indian	A	84·3	84·8	85·6
		B	85·8	84·2	83·8
		C	85*	85	85·9
		D	83·6	84·2	
Technical	Australian	A	98·3	98·3	99·0
		B	99·3	97·9	96·6
		C	98*	98	97·2
		D	98·0	95·6	95·7
Technical	South American	A	86·7	90·0	89·7
		B	86·2	91·7	92·2
		C	89·7*	89	
		D	88·1	87·3	87·9
Technical	South African	A	74·4	74·7	75·0
		B	74·9	74·1	73·8
		C	75*	76·5	76·6
		D	74·3	73·5	74·9
Dispersible powder ..	British	A		86·2	86·0
		B		85·5	86·7
		C		87	86·5
		D		87·8	83·8
Dispersible powder ..	British	A		75·5	76·0
		B		75·2	76·8
		C		78	75·4
		D		76·4	75·0
Dispersible powder ..	Irish	A		79·3	78·4
		B		77·7	78·4
		C		80	79·7
		D		79	78·1
Dispersible powder ..	South African	A		83·2	84·2
		B		82·8	82·4
		C		84	85·5
		D		84·3	82·4

TABLE IV—*continued*

Liquid	.. ..	British	A	35·6	
			B	37·7	
			C	39	38·0
			D	39·2	
Liquid	.. ..	British	A		41·4
			B	38·0	38·6
			C	39·7	37·0
			D	39·3	
Liquid	.. ..	British	A		42·3
			B	39·4	40·9
			C	41·4	40·6
			D	41·5	

* With diethyl ether as solvent.

Appendix I

MODIFIED PARTITION CHROMATOGRAPHIC METHOD FOR THE ASSAY OF PHENOTHIAZINE

APPARATUS—

Chromatographic column—700 mm in length and 22 mm internal diameter, fitted with a coarse sintered disc and tap.

Fraction collector—Fitted to take a 10·0-ml syphon tube.

REAGENTS—

Prepared Celite—Transfer 500 g of Celite 545 to a 3-litre beaker and add 2 litres of concentrated hydrochloric acid. Stir the mixture to an even paste, and set aside with occasional stirring during 12 hours. Decant off the bulk of the hydrochloric acid and add 1 litre of water. Filter the contents of the beaker through a large Buchner funnel lined with a triple layer of Whatman No. 1 filter-paper and wash the residue with water until it is free from acid. Continue the washing, using 500 ml of methanol followed by 1 litre of a mixture of equal volumes of methanol and ethyl acetate. Dry the residue in an oven at 100° C until it is free from solvent odour. Store the prepared Celite in well stoppered glass jars until required.

Hexane—Commercially available hexane of the grade marketed as low in aromatic hydrocarbons normally requires no further treatment. Should the optical density of a 1-cm layer measured against air at 253 nm exceed 0·7, treatment to remove aromatic hydrocarbons is recommended and can be accomplished as described below.

Stir 10 litres of hexane with 500 ml of oleum (20 per cent. of sulphur trioxide) in a 20-litre separator fitted with a glass or stainless-steel paddle for 30 minutes. Allow the layers to separate and run the oleum layer slowly into a vessel containing crushed ice. Wash the hexane layer successively with two 1000-ml portions of water, two 1000-ml portions of 5 per cent. sodium hydrogen carbonate solution and finally two 5000-ml portions of water. Dry the solvent layer over calcium chloride, filter and distil, collecting the fraction boiling between 67° and 69° C.

Acetonitrile—Some samples of acetonitrile are suitable for use as received, but most require the treatment described below to remove basic impurities. Occasional samples have been observed to smell strongly of a nitrobenzene-like substance and have shown no improvement on resin treatment. Such samples should be discarded.

Slurry about 30 ml of Amberlite IR-120(H) with acetonitrile and pour it into a 700 mm × 22 mm chromatographic column fitted with a loose cotton-wool plug. Allow the acetonitrile to drain under gravity until only about a 5-mm layer remains above the surface of the settled resin bed, and reject the acetonitrile. Fill the column with acetonitrile and maintain a head of liquid sufficient to produce a flow-rate from the column of about 10 to 20 ml minute^{-1}. Collect the eluate in a dark glass bottle. The resin-treated acetonitrile can be tested by means of a moistened piece of universal indicator paper held in the mouth of the collecting vessel. Material that does not give an alkaline reaction is suitable for use. Badly contaminated samples may require a second resin treatment to render them suitable.

PROCEDURE—

(*a*) *Preparation of solvent system*—Transfer to a suitable separating funnel 200 ml of acetonitrile and 2000 ml of hexane. Shake the funnel vigorously for 3 minutes and then

allow the layers to separate (the upper layer is the eluent phase and the lower layer the stationary phase). The equilibrated phases should be separated immediately before use.

(*b*) *Preparation of sample and standard*—Dissolve an accurately weighed amount of about 0·1 g of Authentic Specimen phenothiazine* in about 90 ml of eluent phase contained in a 100-ml calibrated flask, and dilute to volume with eluent phase. In a similar manner prepare a second solution containing 0·1 g of the sample. Dilute 10·0-ml portions of both sample and standard solutions in separate 100-ml flasks to 100 ml with eluent phase. Transfer a 20-ml portion of each of the secondary dilutions to separate 100-ml calibrated flasks and dilute both to 100 ml with eluent phase.

(*c*) *Preparation of chromatographic column and subsequent treatment*—Mix 25 g of prepared Celite with 12·5 ml of stationary phase in a 250-ml beaker and transfer the mixture to the chromatographic column in portions not greater than about 3 g, packing down firmly with a tamper after each successive addition. Add about 100 ml of eluent phase to the column and allow it to percolate through until the surface of the mobile phase is 2 to 3 mm above the column packing. Reject any eluate.

TABLE V

EXPERIMENTAL DATA FOR A DETERMINATION BY THE PROPOSED METHOD

Fraction no.	Standard absorption at 253 nm (weight applied 0·1153 mg)	Sample absorption at 253 nm (weight applied 0·0998 mg)
1	0·111	0·089
2	0·042	0·027
3	0·019	0·020
4	0·028	0·017
5	0·021	0·019
6	0·020	0·021
7	0·013	0·011
8	0·013	0·010
9	0·011	0·010
10	0·011	0·011
11	0·011	0·010
12	0·011	0·010
13	0·011	0·010
14	0·011	0·010
15	0·011	0·011
16	0·031	0·055
17	0·268	0·301
18	0·878	0·711
19	0·961	0·601
20	0·431	0·196
21	0·093	0·035
22	0·019	0·014
23	0·010	0·014
24	0·010	0·014
25	0·010	0·014
26	0·010	0·010
27	0·010	0·010
28	0·010	0·010
29	0·010	0·010
30	0·010	0·010

Sum of O.D. under sample band = sum of fractions 16–21

= 1·899 − residual absorption in fractions 16–21 = 6 × 0·010 = 0·060

Net absorption = 1·899 − 0·060 = 1·839

Net O.D. mg^{-1} of sample = 1·839/0·0998 = 18·43

Sum of O.D. under standard band = sum of fractions 16–22

= 2·681 − residual absorption in fractions 16–22 = 7 × 0·010 = 0·070

Net absorption = 2·681 − 0·070 = 2·611

Net O.D. mg^{-1} of standard = 2·611/0·1153 = 22·65

$$\text{Percentage purity of sample} = \frac{18{\cdot}43}{22{\cdot}65} = 81{\cdot}3$$

* Obtainable from the Pharmaceutical Society of Great Britain.

Transfer a 5·0-ml portion of the phenothiazine standard solution (equivalent to 0·1 mg of phenothiazine) to the column, and allow it to percolate through until the level of the solution is within 2 to 3 mm of the top of the column packing. Add successively three 10-ml portions of eluent phase, allowing each to drain to within 2 to 3 mm of the surface of the column packing before applying the next.

Carefully add eluent phase to establish a 40-cm head of liquid above the surface of the stationary phase and maintain this level subsequently (if necessary, limit the flow of eluate from the column to about 5 ml minute^{-1}). Collect thirty-five successive 10-ml fractions of eluate in 6-inch × 1-inch glass-stoppered test-tubes. Measure the optical density of each fraction in 1-cm silica cells by means of a suitable spectrophotometer at 253 nm against eluent phase in the reference cell. Repeat the chromatogram on a fresh column using 5·0 ml of sample solution in place of the standard. Plot the results graphically and assess the mean base-line. Subtract the appropriate base-line correction from each optical density reading under the phenothiazine bands and summate the net optical densities for standard and sample. Then the percentage of phenothiazine in the sample

$$= \frac{\text{Sum of net optical density under sample band}}{\text{Sum of net optical density under standard band}} \times \frac{\text{Weight of standard, mg}}{\text{Weight of sample, mg}} \times 100.$$

A set of experimental figures is given in Table V and serves to illustrate the method of calculation.

Appendix II

Infrared method for the assay of phenothiazine

Procedure—

Weigh 0·1 g of powdered sample into a weighing bottle. By means of a pipette add 5 ml of carbon disulphide, insert the stopper and shake the bottle vigorously for 2 minutes. Fill a 1-mm infrared cell, by means of a syringe, with the sample solution, the solution being expressed through a plug of cotton-wool. Prepare a standard solution from Authentic Specimen phenothiazine in the same way.

Record the infrared spectra of the standard and sample solutions over the range 950 to 890 cm^{-1}.

Construct a base-line, parallel to the 100 per cent. transmission line, passing through the minimum at about 895 cm^{-1} and calculate the absorbance of the peak appearing at 922 cm^{-1}.

$$\text{Then percentage of phenothiazine} = \frac{\text{Absorbance (sample) at 922 cm}^{-1}}{\text{Absorbance (standard) at 922 cm}^{-1}} \times 100.$$

Repeat the above procedure, but take 0·05 g of sample and of standard, record the spectra over the range 1350 to 1200 cm^{-1}, construct a base-line (parallel to the 100 per cent. transmission line) passing through the minimum at about 1210 cm^{-1} and calculate the absorbance of the peak appearing at 1260 cm^{-1}.

$$\text{Then percentage of phenothiazine} = \frac{\text{Absorbance (sample) at 1260 cm}^{-1}}{\text{Absorbance (standard) at 1260 cm}^{-1}} \times 100.$$

Appendix III

Recommended method for the assay of phenothiazine and for the determination of the phenothiazine content of dispersible powders and liquid preparations by gas chromatography

Chromatographic conditions—

All members of the Panel used either a Pye series 104, model 24, or a Perkin-Elmer F11 gas chromatograph with flame ionisation detector, and the chromatographic conditions specified have been found to be optimum for these instruments. Other instruments and detectors can undoubtedly be used, but optimum conditions for the determination should first be established. In the Panel's work, column efficiencies relative to the phenothiazine peak were

about 1900 theoretical plates, giving a resolution factor between phenothiazine and the internal standard of about 3·3.

Support phase:	Chromosorb G, acid washed, DMCS treated, 60 to 85 mesh
Stationary phase:	3 per cent. QF1
Column length—	
Pye:	5 feet
Perkin-Elmer:	6 feet
Column diameter:	4 mm (internal)
Column temperature:	200° C
Inlet temperature:	200° C
Gas flow—	
Nitrogen { Pye:	60 ml minute^{-1}
Nitrogen { Perkin-Elmer:	50 ml minute^{-1}
Hydrogen:	60 ml minute^{-1}
Air:	750 ml minute^{-1}
Detector:	Flame ionisation
Attenuation:	5×10^3 for about 70 per cent. of full-scale deflection.

PREPARATION OF SOLUTIONS—

Standard—Prepare in acetone a solution containing about 0·1 per cent. w/v of Authentic Specimen phenothiazine and about 0·08 per cent. w/v of dibutyl phthalate.

Test—For solid samples prepare a solution in acetone containing about 0·12 per cent. w/v of the phenothiazine under test and about 0·08 per cent. w/v of dibutyl phthalate.

For liquid samples disperse an amount of the liquid calculated to give a concentration of about 0·12 per cent. w/v of phenothiazine in the final solution in 250 ml of acetone. Shake, allow it to settle and centrifuge or filter if necessary. Add sufficient dibutyl phthalate to give a concentration of about 0·08 per cent. w/v.

PROCEDURE—

Apply a 5-μl portion of the test solution to the chromatograph, and measure the areas of the two components. The retention time of dibutyl phthalate is about 2½ minutes and that of phenothiazine about 4 minutes. A further 4 minutes should be allowed to elapse before the next sample is applied, as there is frequently a small impurity following the phenothiazine peak.

Repeat the procedure with a 5-μl portion of the standard solution.

CALCULATION—

$$\text{Percentage of phenothiazine in sample} = \frac{A_1}{A_3} \cdot \frac{A_4}{A_2} \cdot \frac{W_3}{W_1} \cdot \frac{W_2}{W_4} \times P$$

where A_1 = area under the peak relating to phenothiazine for the sample solution;
A_2 = area under the peak relating to phenothiazine for the standard solution;
A_3 = area under the peak relating to dibutyl phthalate for the sample solution;
A_4 = area under the peak relating to dibutyl phthalate for the standard solution;
W_1 = percentage concentration of sample in sample injection solution;
W_2 = percentage concentration of standard in standard injection solution;
W_3 = percentage concentration of dibutyl phthalate in sample injection solution;
W_4 = percentage concentration of dibutyl phthalate in standard injection solution;
P = percentage purity of the standard phenothiazine.

Appendix IV

COMPARISON OF THE RECOMMENDED METHOD WITH ANOTHER PUBLISHED METHOD

After the Panel had completed its work on the development of its recommended gas-chromatographic method for determining phenothiazine, details of the method proposed by Bramlett[5] were published. In this method a solution of the sample in chloroform is chromatographed on a column of 5 per cent. Apiezon L on 100 to 120-mesh Gas Chrom Q at 220° C.

The detector used is flame ionisation, and promethazine hydrochloride is used as internal standard. As this method was under investigation by the A.O.A.C. for possible acceptance as an official method, the Panel decided to carry out a comparison of results by the two methods. Owing to non-availability of samples in one laboratory, only three laboratories were able to take part in this work and the results are shown in Table VI.

From Table VI it can be seen that there is little difference between the results obtained by the two methods. Members did, however, consider that the retention time of promethazine hydrochloride (15 to 20 minutes) was unduly long for use in a method for which it was normal practice to carry out duplicate injections. Also, the use of chloroform as solvent was subject to the same disadvantages as were met with methylene chloride, which the Panel had earlier rejected as a solvent, and some difficulty was experienced with liquid preparations in dispersing the phenothiazine in chloroform.

TABLE VI

COMPARISON OF RESULTS BY BRAMLETT'S METHOD AND BY THE METHOD RECOMMENDED IN APPENDIX III

Sample	Origin	Laboratory	Phenothiazine found, per cent. w/w, by Bramlett's method[5]	Phenothiazine found, per cent. w/w, by Recommended method
Technical ..	.. Unknown	A	90·3, 91·3	93·0
		B	91·7	92·5
		C	91·4, 93·0	91·4
Technical ..	.. British	A	91·9, 91·3	91·5
		B	91·1	90·9
		C	91·9, 92·7	90·7
Technical ..	.. Indian	A	85·9, 85·9	85·6
		B	84·5	83·8
		C	85·6, 85·9	85·9
Technical ..	.. South American	A	91·1, 90·4	89·7
		B	90·0	92·2
Technical ..	.. South African	A	79·4, 80·1	75·0
		B	76·1	73·8
		C	75·4, 81·7	76·6
Technical ..	.. Australian	A	97·6, 97·4	99·0
		B	97·3	96·6
Dispersible powder	.. British	A	88·8, 87·6	86·0
		B	87·4	86·7
Dispersible powder	.. British	A	77·6, 77·7	76·0
		B	75·7	76·8
Dispersible powder	.. Irish	A	78·6, 80·0	78·4
		B	78·7	78·4
Dispersible powder	.. South African	A	85·7, 86·5	84·2
		B	84·8	82·4
		C	85·5	85·5
Liquid ..	.. British	B	37·1	38·6
Liquid ..	.. British	B	42·6	40·9

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Book Reviews

HORMONES IN BLOOD. Edited by C. H. GRAY and A. L. BACHARACH. Second Edition. Volume 1. Pp. xviii + 577. London and New York: Academic Press. 1967. Price 130s.; $22.50.
HORMONES IN BLOOD. Edited by C. H. GRAY and A. L. BACHARACH. Second Edition. Volume 2. Pp. xviii + 686. London and New York: Academic Press. 1967. Price 150s.; $26.

I imagine that when the above editors first considered being responsible for the compilation of these volumes, they did not fully realise the extent to which they had committed themselves.

It is a vast subject, sub-divided into very many specialisations. They considered it time that the mass of existing publications concerning the levels of some fifteen hormones in human blood or plasma in health and disease should be critically surveyed. The magnitude of the task only revealed itself subsequently. The first difficulty was to find suitable experts in the particular specialisations. These experts were asked to correlate the present knowledge on plasma hormone levels in health and disease, also to assess critically the techniques by which published methods have been obtained, and to re-assess the value of formerly recorded levels in the light of more current technical advances. In choosing the authors for the various sections of the book, the editors have called upon the services of experts from the other side of the Atlantic, but have confined themselves to English writing contributors. These contributors were asked to provide accounts and descriptions of analytical methods available, and at the same time to outline the biochemistry, metabolism and physiological effects of the various hormones when comparing normal and abnormal conditions.

In the introductory chapter, the editors discuss the various methods of measurement of hormones in blood and point out that, in the main, the past bio-assays have been the most frequently used. They point out, however, that with the increasing knowledge of the structural formulae of the various hormones and the advances in technology, in the future undoubtedly chemical methods will tend to replace bio-assay techniques; this is becoming already increasingly apparent.

In subsequent chapters on procedure, there are two very well documented contributions relating to gel filtration and immuno-assay of hormones. The range of hormones dealt with in this volume is quite extensive, and the individual subjects have been surveyed in every instance in great detail. Although there are not many chemical methods described, all the procedural technique is of considerable interest to biochemists; in particular, the methods of isolation of the specific hormones prior to determination. In many instances this aspect of the book is so detailed that it is worth a study by those chemists in other fields, who are interested in the separation of small quantities of substances from complex biological material, prior to final determination.

As might be expected, a considerable portion of the volume is given over to the pituitary hormones and associated gonadotrophins. In these chapters there is much information about methods of separation prior to bio-assay, which has been correlated for the first time. The most extensive chapter in the book is on the iodine-containing hormones. This chapter finishes with about 800 references and is undoubtedly the most thorough publication on this subject to date.

The second volume is largely taken up by the steroid hormones, and again the subject is dealt with in great thoroughness under the respective headings. With this group, chemical methods of determination are much more the order of the day, and there is an excellent general chapter on the use of gas chromatography as a tool in this field. Gas chromatography, thin-layer chromatography and similar procedures as applied to the determination of the individual steroid substances are preferred, but colorimetric chemical methods have also been described. This volume also includes a survey of the catecholamine group of hormones, which have been more recently of considerable clinical interest.

The final chapter is on parathormone and associated substances, about which there is still much to be learned. However, assay methods have been described and limits in health and disease have been outlined.

The characteristic feature of the two volumes is the care with which they have been prepared, and the great attention that has been paid to the correct use of the English language, despite difficulties inevitable when contributors from both sides of the Atlantic are involved. As one would have expected from Alfred Bacharach, the editing has been carried out with meticulous care. His imprint on these volumes is very apparent. As his co-editor, Dr. Gray states that his

death in 1966 was a loss to science and the scientific literary world, and Dr. Gray freely admits the debt that he owes to him for an appreciation of the problems and practice of editing. On reading through the second volume (which was largely edited by Dr. Gray) it is quite clear that he has gained much from his association with Alfred Bacharach. R. F. MILTON

COMPLEXOMETRY WITH EDTA AND RELATED REAGENTS. By T. S. WEST, D.Sc., Ph.D. Pp. 235. Poole, Dorset: B.D.H. Chemicals Ltd. 1969. Price 50s.

This book is the third (and greatly expanded) edition of a small booklet written by T. S. West and A. S. Sykes and first published about 1957. The second edition, written by T. S. West, appeared in 1960 and differed from the first in that more recent developments were included in an appendix. In the present edition this material has now been promoted to individual chapters and has been expanded and up-dated.

When the first edition appeared, there was virtually no collected information on complexometry available in the English language and the little book met a real need. Today there are many books on complexometry available in English, so that it might be thought that the original need has long since passed. This may well be so, but the field of complexometry is so extensive that there is still room for further books. Even if there were a plethora of books on this subject, the present one would take its place in the front rank. Not only does it cover all the most important aspects of the subject, but it is written crisply and concisely, so that apart from keeping the number of pages to a minimum, it is remarkably easy to find any particular information that is required. The author has had extensive practical experience in the field, which fortifies his recommendations when a personal choice is made.

The book is not without its blemishes and some are remarkable in a book of otherwise high quality. For example, it is startling to find the author reverting to the use of the term "complexone," a registered trade name, and his avoidance of recommended I.U.P.A.C. terminology, especially when he was partly responsible for that terminology. It would seem that this is due to incomplete scavenging of the older editions. Many authors fall into this trap and it can probably only be avoided completely by wholly re-writing the new edition. On the other hand, for a very busy man the choice of the one or the other may make the difference between a new edition or none at all.

The curious view is advanced that the formation of a precipitate to remove an unwanted ion, "does not constitute a true masking action since a physical (*i.e.*, phase) separation occurs. . . ." The purpose of masking is to remove an unwanted ion from the sphere of reaction so that it is unnecessary to apply a separation method. If this can be accomplished then the ion is said to be "masked." The manner in which this is achieved is immaterial; it is only the final effect that is important. Perhaps a more important argument is that the term "masking" was coined by Feigl who, as the inventor, has the right to define his own term or concept, and he has always included precipitation amongst methods of masking, provided that the further reaction takes place in the presence of the precipitate.

The author is not correct in stating that only two examples of complexometric titration were known until after the close of the war; *i.e.*, silver with cyanide and chloride with mercury(II). There were others, and particularly noteworthy was the introduction of sodium diethyldithiocarbamate as a titrant for platinum and palladium by Pollard in 1938. This reagent adumbrated complexometry as we know it today.

Apart from these faults this is an excellent and absorbing book, bristling with valuable information; it is good value at the price and most chemists should find it both interesting and useful.

R. BELCHER

TECHNIQUE OF ORGANIC CHEMISTRY. Edited by E. S. PERRY and A. WEISSBERGER. VOLUME XIII. GAS CHROMATOGRAPHY. By ORION EDWIN SCHUPP III. Pp. xxii + 437. New York, London, Sydney and Toronto: Interscience Publishers, a division of John Wiley & Sons. 1968. Price 155s.

The book is a member of the series on techniques of organic chemistry, and should do much to enhance the reputation of this series. The vitally important rôle that gas chromatography now plays as an analytical technique would have been difficult for anyone but the fanatic to predict. As the number of papers on this topic now occupies, on average, one third of analytical papers published, books on gas chromatography are needed.

In the last 12 months, the "third generation" of books has begun to be published. These books should highlight the salient features of the technique, so that a beginner might learn sufficiently quickly to make progress with the technique, while experienced workers could savour the comments made in the light of their own experience. The present book is really directed at the practising gas chromatographer and emphasises operation, design of equipment, the properties of stationary-phase materials and volatile derivative preparation, together with details of specialised and auxiliary techniques.

The book is divided into seven chapters, each giving a broad account of the area of specialisation described. The titles of the chapters are Introduction; Theory of Gas Chromatography; Operation, Design and Performance of Equipment; Columns; Special Chromatographic Techniques; Qualitative Analysis; and Quantitative Analysis. One of the major problems in gas chromatography, with the large number of references and literature available, is to make effective use of the application of gas chromatography when applied to a problem. The variety of problems that can be solved by gas chromatography is wide and, like all instrumental analysis, the scope is limited by the equipment available to the research worker. The intention of this book is to help the research worker make a judicious choice of the parameters to be exploited for his particular case.

Mention should be made about some of the chapters, because it is in this respect that the present book differs from its contemporaries. Chapter 1 is no different from other introductions, it covers the ground adequately and clearly and all of the terms and parameters are defined.

In Chapter 2 the theory of gas chromatography is obviously not necessary to carry out chromatography successfully, but a deepening knowledge of the theory is needed if we are to continue to achieve satisfactory results with increasingly difficult separations. This chapter is recommended because it gives, in succinct form, the theories currently held, and because of its honesty in admitting that expressions describing peak broadening factors are "virtually impossible to derive because of the fact that many parameters are averages of a large number of possibilities."

In the third chapter, the various parts of a gas-chromatographic determination are discussed with reference to the usefulness of the separations that are to be achieved. To the reviewer the number of detectors described is only a minimum, but to others this may be a challenge.

Chapter 4 discusses, under the heading Columns, both the solid support and the stationary phase. Some salient points are made in this chapter, often ones that we neglect to our cost, *e.g.*, the inertness or otherwise of the support material. The second part of the chapter discusses the general requirements of the liquid phase, the temperature ranges over which a liquid can be used and the construction of "tailor-made" or mixed-phase columns. However, as is inevitable, this chapter looks a little dated with the development in phases over the last 2 years.

The object of the fifth chapter is to highlight the trends in the processes of derivatisation, so that normally involatile compounds can be gas chromatographed. With the ever-increasing use of the technique in biochemistry, these processes will receive increasing attention. The chapter discusses the processes needed for the derivatisation of acids, esters, fats, alcohols, phenols, amines and polymers.

The last two chapters discuss qualitative and quantitative analyses. To a major extent, these are conventional chapters but are clear and well written.

In the reviewer's experience students find little difficulty in understanding the relevant chapters, and the book is to be recommended, the only pity being its high price, but perhaps this is the price to be paid for quality.

G. NICKLESS

Errata

JUNE (1969) ISSUE, p. 500, 14th line of text. *For* "catalyst B–3–11" *read* "Catalyst R3–11."

JULY (1969) ISSUE, p. 523. Replace equation (1) with

$$IO_4^- + 2\,[Fe(1,10\text{-phen.})_3]^{2+} + 2H^+ \rightarrow IO_3^- + 2\,[Fe(1,10\text{-phen.})_3]^{3+} + H_2O \quad (1).$$

APPOINTMENTS VACANT

Daniel C. Griffith & Co. Ltd. are looking for a Chief Chemist to take charge of their analytical laboratories and staff of 60 chemists. The work covers the entire mineral field from precious metals and rare earths to base metals (ferrous and non-ferrous).

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UNIVERSITY OF READING, Department of Agriculture. Analyst required for Analytical Section now under construction at Earley Gate. H.N.C., or O.N.C. studying for H.N.C. preferred. Salary in the range £773 – £1077 (or £1127 for H.N.C.) according to age, qualifications and experience. Suitable person encouraged to study to professional level. Apply in writing, quoting M.53 to Assistant Bursar (Personnel), University of Reading, Reading, Berks.

The Determination of Silicon in Intact Steel Samples with a Low Output Neutron Generator

A low output, sealed-tube neutron generator has been used to determine the silicon content of steels, counting and flux monitoring being carried out with simple equipment.

T. B. PIERCE and K. HAINES

Analytical Sciences Division, A.E.R.E., Harwell, Near Didcot, Berks.

Analyst, 1969, **94**, 886–887.

Nitrate Determination in Soil Extracts with the Nitrate Electrode

This paper deals with investigations on nitrate determination with a nitrate-specific ion electrode. Analytical results for the nitrate content in soil extracts are compared with analyses carried out by two colorimetric methods. The extracting agents used are 0·02 N copper sulphate, 2 N potassium chloride and distilled water. Results for nitrate determinations in soil suspensions and corresponding filtrates are also compared.

The stability of soil extracts, the influence of variation in the ratio of grams of soil to millilitres of extracting agent and in the shaking time, interferences caused by ionic strength and anions, accuracy and reproducibility are also discussed.

The investigation shows that nitrate determination with the electrode is reliable both for soil extracts and for soil suspensions. The determination is rapid, and the interferences are not serious.

A. ØIEN

Institute of Soil Science, Agricultural College of Norway, Vollebekk, Norway

and A. R. SELMER-OLSEN

Chemical Research Laboratory, Agricultural College of Norway, Vollebekk, Norway.

Analyst, 1969, **94**, 888–894.

Automated Distillation Procedure for the Determination of Nitrogen

A distillation step has been incorporated into the automated determination of ammonia by the indophenol method.

By this means the application of the method has been extended, and interference from metal ions and the need for dialysis of turbid solutions have been eliminated. The reliability of the method has been improved without loss of speed.

J. KEAY and P. M. A. MENAGE

CSIRO Division of Soils, W.A. Laboratories, Private Bag, P.O., Wembley, Western Australia.

Analyst, 1969, **94**, 895–899.

Dual Column and Derivative Techniques for Improved Specificity of Gas - Liquid Chromatographic Identification of Organochlorine Insecticide Residues in Soils

Derivatives of organochlorine insecticides exhibiting R_t values substantially different from those of the parent insecticides were obtained for *pp'*-TDE, *pp'*-methoxychlor, *op'*-DDT and *pp'*-DDT on treatment with 2 per cent. ethanolic potassium hydroxide, and for endrin and dieldrin with ethanolic concentrated hydrochloric acid; partial clean-up of soil extracts was achieved by the alkali treatment. The use of dual columns, namely, DC-200 and diethylene glycol succinate, in conjunction with derivative formation, provided R_t values sufficiently different for improved organochlorine insecticide identification with minimal additional effort.

H. B. PIONKE, G. CHESTERS and D. E. ARMSTRONG

Department of Soil Science, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

Analyst, 1969, **94**, 900–903.

The Spectrophotometric Determination of Riboflavine in Urine

A simple spectrophotometric method for the determination of riboflavine in urine is described. The urine, after treatment with zinc acetate and formaldehyde, is passed through a small column of large particle-size talc on which the riboflavine is selectively and quantitatively adsorbed. Successive washings with 0·01 N hydrochloric acid and 5 per cent. dioxan remove the urinary pigments and other urinary constituents almost completely. The riboflavine eluted with 20 per cent. dioxan is measured spectrophotometrically at 444 nm. Some results are given for the rate of excretion of riboflavine in urine following intravenous injection of the vitamin.

NAGI WAHBA

Biochemistry Department, Faculty of Medicine, Ain Shams University, Cairo, U.A.R.

Analyst, 1969, **94**, 904–908.

The Analysis of Mixtures of Methyl Ethanesulphonate and Ethyl Methanesulphonate by a Differential Reaction Rate Method

Mixtures of methyl ethanesulphonate and ethyl methanesulphonate were determined by the differential reaction rate method. Suitable reaction rates for the analysis were given by the solvolysis of the mixture in butanol at 100° C.

A. J. W. BROOK and K. C. MUNDAY

Ministry of Defence (A.D.), Chemical Defence Experimental Establishment, Nancekuke, Redruth, Cornwall.

Analyst, 1969, **94**, 909–911.

A Simple Trace Reader with Digital Print-out

The construction and performance of an inexpensive, manually operated, electromechanical trace reader is described. Function generator circuits involving the use of biased diode networks are used to simulate convex and sigmoid calibration graphs by a series of linear approximations. The outputs of these circuits are used to drive a digital voltmeter with print-out. Comparative trials of the conventional manual procedure and the trace reader are reported. A measurement rate of 30 peaks minute^{-1} and precision of 0·5 per cent. f.s.d. are readily achieved with the trace reader. The system is less demanding on the powers of concentration of the operator, reduces the risk of transcription error and doubles the measurement rate of a single operator.

J. B. DAWSON

Department of Medical Physics, University of Leeds, The General Infirmary, Leeds 1.

R. MILNER and D. MAWSTON

Medical Research Council, Mineral Metabolism Research Unit, The General Infirmary, Leeds 1.

Analyst, 1969, **94**, 912–917.

Indicator-tube Method for the Determination of Benzene in Air

A linear colorimetric method for determining benzene vapours in air is described. The method is based on a colour reaction between the benzene vapours and 0·200-mm silica gel treated with 0·7 g ml^{-1} of a 5 per cent. solution of cerium sulphate in fuming sulphuric acid. The glass tubes are filled with indicator powder to a length of 50 mm. The indicator tubes are calibrated experimentally for benzene concentrations of from 0 to 0·3 mg l^{-1} when 500 ml of air are aspirated, and from 0 to 2·0 mg l^{-1} when 100 ml of air are aspirated. The accuracy of the method is from ± 15 per cent. for low concentrations of benzene and ± 5 per cent. for high concentrations of benzene. The sensitivity is 0·005 mg l^{-1}. The method is not applicable in the presence of toluene and xylene.

P. KOLJKOWSKY

Transport Medical Institute, Sofia, Bulgaria.

Analyst, 1969, **94**, 918–920.

The Detection of Prophylactic Drugs in Animal Feeding Stuffs by Thin-layer Chromatography

A procedure is described for detecting the presence of seventeen prophylactic drugs commonly present in animal feeding stuffs. Only two solvent systems are used for separation purposes. The drugs investigated were: acinitrazole, 2-chloro-4-nitrobenzamide (aklomide), aminonitrothiazole, amprolium, N^4-acetyl-4′-nitrosulphanilanilide (APNPS), buquinolate, decoquinate, dimetridazole, 3,5-dinitrobenzamide (DNBA), ethopabate, furazolidone, clopidol (metichlorpindol), nitrofurazone, pyrimethamine, methyl benzoquate, sulphaquinoxaline and 3,5-dinitro-*o*-toluamide (zoalene).

P. W. HAMMOND and R. E. WESTON

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1969, **94**, 921–924.

The Determination of Dimetridazole in Animal Feeds

Report prepared by the Prophylactics in Animal Feeds Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W.1.

Analyst, 1969, **94**, 925–931.

The Assay of Phenothiazine

Report prepared by the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods for the Evaluation of Drugs.

Joint Committee of the PHARMACEUTICAL SOCIETY and the SOCIETY FOR ANALYTICAL CHEMISTRY

9/10 Savile Row, London, W.1.

Analyst, 1969, **94**, 932–941.

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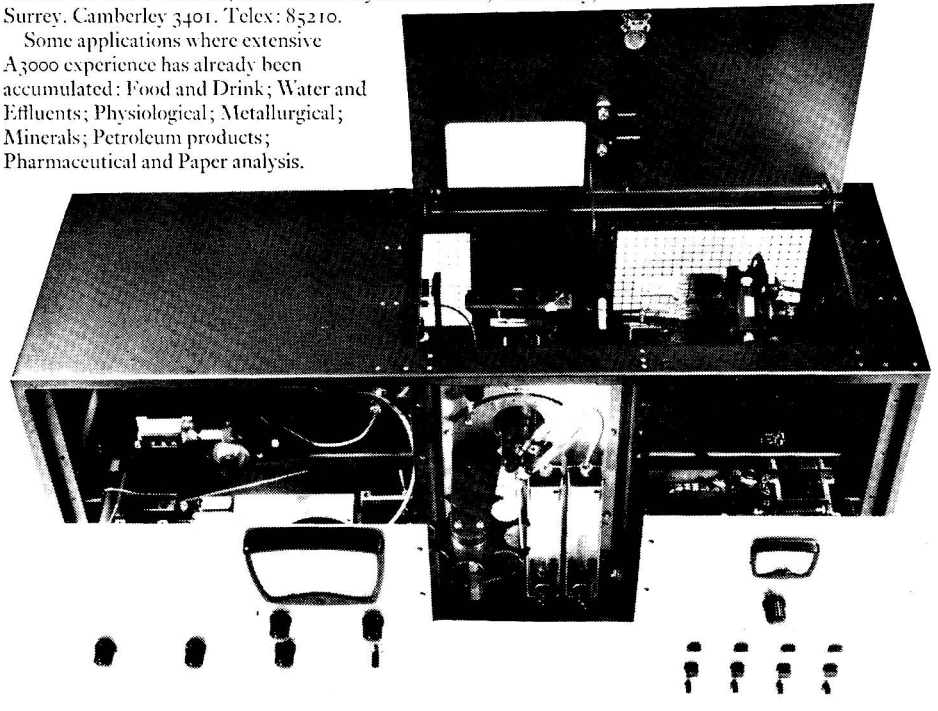

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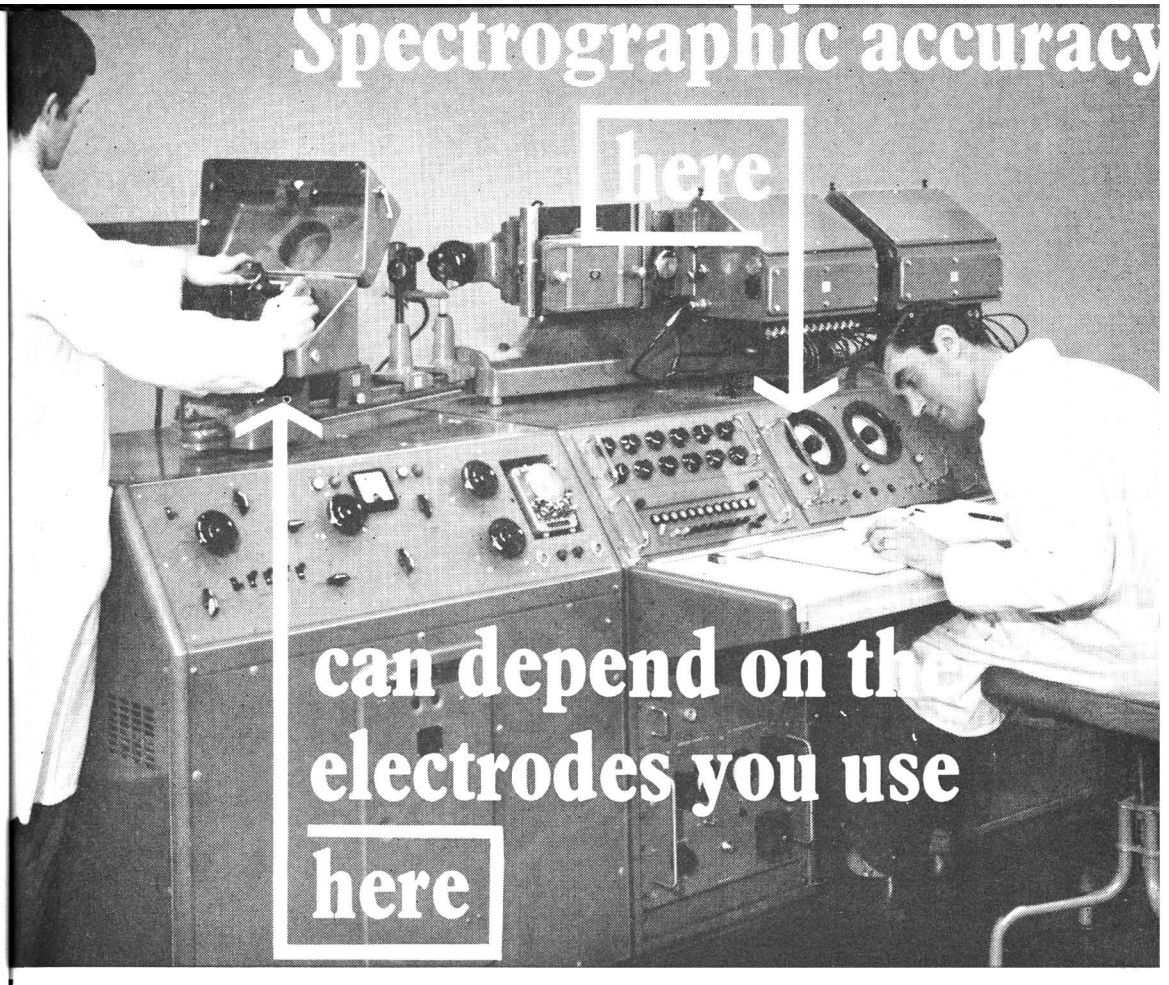

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